Supporting Information

MATERIALS AND METHODS

Lentiviral infection of MiaPaCa2 cells with 6HF-ubiquitin like constructs. A tandem 6His and Flag tag was introduced into empty pCCL-WPS-mPGK lentiviral vector, at the 5' end of the multi-cloning sites portion, to produce the pCCL-6HF vector. The full-length cDNA for human Ubiquitin, Nedd8, and SUMO1, were subcloned into this vector using SmaI and EcoRV restriction sites for Ubiquitin, BamHI and EcoRV for Nedd8, and BamHI for SUMO1. Each plasmid was verified by DNA sequencing. Lentiviral particles were generated by transfecting 293T cells with a mix of 1/3 pCCL construct (Ub, Nedd8, SUMO1, or GFP), 1/3 ΔHelper (carries sequence necessary for viral assembly of lentivirus) and 1/3 pVsVg (expresses the vesicular stomatitis virus envelop glycoprotein G pseudotype), using Lipofectamine reagent (Invitrogen) and following manufacturer's recommendations. 24 hours post-transfection, the medium has been changed for fresh one. 24 hours later, medium has been changed again and viruses containing medium was collected, filtered through a 0.2 µm filter, and added on 40% confluent MiaPaCa2 cells seeded in 25 cm^2 flasks. This step has been repeated 24 hours later to perform a second infection. Five days after infection, expression of GFP was verified by fluorescence microscopy and Ubiquitin, Nedd8, and SUMO1 expression controlled by western blot.

Transient transfection. HEK-293T and MiaPaCa2 cells were transiently transfected using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's instructions. Briefly, 80% confluent cells seeded in 10 cm culture dishes were transfected using a mix of 6 µg of DNA and 18 µL of Lipofectamine per dish for 4 h. For experiments with Gemcitabine, PD169316 or SB203580 treatments, 1 µg of 6HF-SUMO1 and 1 µg of GST-SNIP1 were transfected. For experiments with the SNIP1 KR mutants, 2 µg of 6HF-SUMO1 and 2 µg of GST-SNIP1 constructs were transfected. In all cases, DNA concentration was normalized to 6 µg using empty pCCL-WPS-mPGK lentiviral vector. Cells were harvested 24 hours post-transfection or after the indicated treatments. For FACS analysis, caspase activity

and MTT assays, MiaPaCa2 cells were transfected using a mix of 12 µg of GST-SNIP1 WT or the indicated KR mutants and 36 µl of Lipofectamine per dish for 4 hours. 24 hours post-transfection, cells were seeded either in 6-well plates with 1 million of cells per well, 24-well plates with 100 000 cells per well or 48-well plates with 50 000 cells per well for FACS, MTT and caspase assays, respectively. Cell proliferation and apoptosis were analyzed as described below.

For siRNA experiments, 60-80% confluent MiaPaCa-ArgBP2 cells in 6 cm diameter dishes were transfected with a mix of 0.5 nmol control or p38-alpha siRNA and 20 µl Effecten transfection reagents (QIAGEN) in a 2.6 ml final volume of OptiMEM for 4 hours. Gemcitabine treatment was applied 24 hours later.

Single step purification of 6Histidine-ubiquitin, Nedd8 and SUMO1 conjugates. Purification of 6His-ubiquitinated, -neddylated and -sumoylated conjugates was performed as described in references (1, 2). Thirty-six hours after gemcitabine treatment, MiaPaCa2 cells seeded in 10 cm culture dishes were washed twice with PBS and scraped in 1 ml of PBS. Twenty per cent of cell suspension was used for direct Western blot analysis (see below). The remainder was lysed in 6 ml of 6 M Guanidinium-HCl, 0.1 M Na_2HPO_4/NaH_2PO_4, 0.01 M Tris/HCl, pH 8.0, 15 mM imidazole and 10 mM β-mercaptoethanol (buffer 1). After sonication of cellular lysates to reduce viscosity, 50 µl of Ni^{2+}-NTA resin (Qiagen) pre-washed with lysis buffer were added and lysates were rotated at room temperature (RT) for 4 h. The beads were successively washed during 5 min at RT with 750 µl of each of the following buffers: buffer 1; buffer 2 (8 M urea, 0.1 M Na_2HPO_4/NaH_2PO_4, 0.01 M Tris/HCl, pH 8.0, 10 mM β-mercaptoethanol); buffer 3 (8 M urea, 0.1 M Na_2HPO_4/NaH_2PO_4, 0.01 M Tris/HCl, pH 6.3, 10 mM β-mercaptoethanol) plus 0.2% Triton X-100; and then buffer 3 plus 0.1% Triton X-100. After the last wash 6His-ubiquitinated, -neddylated and -sumoylated conjugates were eluted by incubating the beads in 50 µl of buffer 4 (200 mM imidazole, 0.15 M Tris/ HCl pH 6.7, 30% glycerol, 0.72 M β-mercaptoethanol, 5% SDS) for 20 min at RT. Eluates were analyzed by Western blot.

Non denaturing cell lysis. For direct Western blot analysis, the cell pellet obtained from the 20% cell suspension described above was lysed in cold phosphate lysis buffer (50 mM NaH_2PO_4, 150 mM NaCl, 1% Tween20, 5% Glycerol, pH 8.0) supplemented with protease cocktail inhibitor (Roche, 1:200), 10 mM N-ethylmaleimide (NEM) and 2 mM phenylmethylsulfonyl fuoride (PMSF). After 5 min of incubation on ice, lysates were centrifuged10 min at 13000 rpm at 4°C and pellets were discarded. Protein concentration in the supernatant was determined using Protein Assay (BioRad), and equal amounts of total protein were loaded for western blot analyses.

Western blot analysis. Proteins were resolved by SDS-PAGE, transferred to nitrocellulose filters, blocked 1h at room temperature, and blotted overnight in Tris-buffered saline / 5% BSA / 0,1% Tween20 with primary antibodies at 1:1000. After extensive washings in TBS / 5% BSA / 0,1% Tween20, filters were incubated 1h at RT with a HRP conjugated secondary antibodies at 1:5000 before being revealed with ECL. Acquisition was performed with a Fusion FX7 imager (Vilber-Lourmat, France). For Flag (6HF-constructs) and β-Tubuline immunoblots, SNAP i.d. protein detection system (Millipore) was used following the manufacturer's instructions.

Immunofluorescence. Parental and transduced MiaPaCa2 cells were seeded on cover slips and incubated at 37°C, 5% CO_2 in a humidified atmosphere. When reaching 50% confluence, cells were fixed in 4% paraformaldehyde in PBS for 5 min, treated with 50 mM NH_4Cl in PBS for 10 min, and permeabilized with 0.2% Triton X-100 in PBS for 3 min. After blocking for 1 h with 5% FBS in PBS, cells were probed with primary antibodies (dilution 1:50 in 5% FBS in PBS for 60 min), washed, and incubated with Alexa fluorophore antibodies (Invitrogen) for 60 min. Preparations were mounted using Fluoromount G, and the images were captured with a Nikon microscope Eclipse 90I.

FACS Analysis. To analyze the impact of SNIP1 on G2/S and sub-G1 phases, MiaPaCa2 cells seeded in 10 cm dishes were transfected with 12 µg of SNIP1 WT, K30R, K108R, or an empty vector as control. 24 hours post-transfection, cells were seeded in 6-well plates with a total of 1 000 000 cells per well. The day after, cells were harvested, stained with propidium iodide and analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Data analysis was performed using FlowJO (Treestar, Ashland, OR) software.

Caspase-3/7 activity assay. MiaPaCa2 cells seeded in 10 cm dishes were transfected with 12 µg of SNIP1 WT, K30R, K108R, or an empty vector as control using Lipofectamine 2000 reagent (Invitrogen). 24 hours post-transfection, cells were seeded in 48-well plates with a total of 50 000 cells per well. 8 hours later, cells were treated or not with 10 µM of Gemcitabine, and Caspase-3/7 activity was measured 72 hours after treatment using Caspase-Glo® 3/7 assay (Promega, USA) following the manufacturer's instructions.

MTT assays. To determine the growth curve and Gemcitabine sensitivity of parental or transduced pancreatic cancer cells, a total of 20 000 cells were plated in 24-well plates. Every 24h, DMEM medium was changed to DMEM medium containing 10% of MTT at 5 mg/ml in PBS. After removal of the medium, 250 or 500 µl of 0.1 M HCl into Isopropanol were added to each well to dissolve the formazan crystals. The absorbance at 540 nm was determined using an Epoch plate reader (Bio-Tek Instruments, Inc, USA). Duplicate wells were assayed for each condition and S.D. was determined. To analyze the impact of SNIP1 SUMOylation on Gemcitabine induced apoptosis, MiaPaCa2 cells seeded in 10 cm dishes were transfected with 12 µg of SNIP1 WT, K30R, K108R, or an empty vector as control. 24h hours post-transfection, cells were seeded in 24-well plates with a total of 100 000 cells per well. 8 hours later, cells were treated or not with 10 µM of Gemcitabine, and after 72 hours of treatment, DMEM medium was changed to DMEM medium containing 10% of MTT at 5 mg/ml in PBS. After removal of the

medium, 250 µl of 0.1 M HCl into Isopropanol were added and the absorbance at 540 nm was determined as before. Triplicate wells were assayed for each condition and S.D. was determined.

iTRAQ labeling. 50 µg of proteins were precipated with 5 volumes of cold acetone for 2h at -20°C, centrifuged for 8 min at 10 000 x g. Proteins were then dissolved in 20 µL of dissolution buffer, denatured, reduced, alkylated and digested with trypsin overnight at 37°C, following manufacturer's protocol (iTRAQ® Reagent Multiplex Buffer kit, Applied Biosystems, Foster City, CA, USA). The ratio enzyme/substrate was 1:10 (w/w) and the pH was checked to ensure a complete digestion. The 2 conditions were triplicated and labelled with the iTRAQ-8plex according to the iTRAQ Reagents Application Kit Plasma (Applied Biosystems). Using the 114, 115, 116, 117, 118 and 119 labels. The content of each iTRAQ reagent-labeled sample was pooled into one tube and cleaned-up using an exchange chromatography (SCX/ICAT cation exchange cartridge, ABsciex, Foster City, USA) and reverse-phase chromatography C18 cartridge (Sep-Pak C18, Waters, France).

iTRAQ labeled peptide fractionation and Mass Spectrometry

Isoelectric point fractionation. Isoelectrical point (pI) fractionation was done with the Agilent 3100 OFFGEL fractionator (Agilent Technologies). Dried peptide samples were solubilized with a carrier-focusing buffer (water/glycerol/ampholyte) according to the manufacturer protocol. The 12 cm long IPG gel strip (Agilent) with a linear 3-10 pH range was rehydrated 15 min. 150 µL of sample was loaded into each of the 12 wells. During the run, peptides were focused at constant intensity of 50 µA, with a typical voltages ranging from 0.5 to 8 kV until the 20 kVh level was reached. After focusing, the 12 peptide-fractions were withdrawn and the wells were washed with 200 µL of 49% water (v/v), 50% methanol (v/v) and 1% TFA (v/v). The wash solution was collected and pooled with the corresponding fraction.

Mass spectrometry. pI-fractions were analyzed on nESI HCD-enabled Orbitrap Velos instrument (Thermo Scientific) connected to an Ultimate® 3000 Rapid Separation LC (Dionex). Upon injection, the fraction was loaded onto the enrichment column (C18 PepMap100, 100 µm id, 100 Å pore size, 5 µm particle size,

Dionex) using 2% ACN, 0.1% FA. After the analytical column (C18 PepMap100, 75 µm id, 100 Å pore size, 2 µm particle size) was switched in-line, the HPG nano pump delivered a 180 min linear gradient of 2% ACN, 0.1% FA (solution A) and 80% ACN, 0.1% FA (solution B) at 300 nL/min flow rate. Instrument method for the LTQ Orbitrap was set up in data dependant mode to switch consistently between MS and MS/MS. The signal threshold for an MS/MS event was set to 500 counts. Charge state screening was enabled to exclude precursors with 0 and 1 charge states. Dynamic exclusion was enabled (exclusion list size 500, exclusion duration 30 s). For internal mass calibration the 445.120025 ions was used as lock mass. MS spectra were acquired with the Orbitrap in the range of m/z 400-1700 at a FWHM resolution of 30 000. The 10 abundant precursor ions were selected and HCD fragmentation was performed in 2 steps at 45% SNCE (Stepped Normalized Collision Energy) in the HCD collision cell with a normalized collision energy width of 10%. This two steps strategy was used to optimize both low mass range for quantification and high mass range for identification. Fragments were then ejected from the HCD cell and read out in the Orbitrap at a FWHM resolution of 7 500. To identify and quantify more proteins in the deep proteome, a second injection was done with an exclusion list. This exclusion mass list was generated using the first series of data containing the m/z and the elution time of identified and quantified peptide with a high confidence.

Protein identification and quantification. Raw files generated from MS analysis were combined and processed with Proteome Discoverer 1.3 (Thermo Fisher Scientific) for quantification and Mascot (version 2.3.0; Matrix Science Inc., London, UK) and Sequest (3) for protein identification. The search was performed against the human reference proteome database containing 88 837 sequences extracted from Uniprot the 28th May 2013. Database search were done using the following settings. Trypsin was chosen for cleavage specificity and a maximum of one trypsin miss-cleavage allowed. Fixed modifications were set up for cysteine alkylation (Methylthio) and iTRAQ label (N-terminal peptide and lysine). Dynamic modifications were allowed for Tyrosine (iTRAQ label), methionine (oxidation), and for Asparagine and Glutamine (Deamidation). A peptide mass tolerance of 6 ppm and a fragment mass tolerance of 0.1 Da were allowed for search analysis. All quantification data were normalized to beta-actin and beta-tubulin. A

protein was considered to be significantly identified when 2 or more high confidence unique peptides were assigned at 5% false discovery rate. Differential proteins were selected with a fold change greater to 1.5.

References

1. Tatham, M. H., Rodriguez, M. S., Xirodimas, D. P., and Hay, R. T. (2009) Detection of protein SUMOylation in vivo. *Nat Protoc* **4**, 1363-1371

2. Xirodimas, D., Saville, M. K., Edling, C., Lane, D. P., and Lain, S. (2001) Different effects of p14ARF on the levels of ubiquitinated p53 and Mdm2 in vivo. *Oncogene* **20**, 4972-4983

3. Jimmy K. Eng, Ashley L. McCormack, and John R. Yates, III (1994). "An Approach to Correlate Tandem Mass Spectral Data of Peptides with Amino Acid Sequences in a Protein Database". *J Am Soc Mass Spectrom* **5** (11): 976–98

Supplementary Figures Legends

Fig. S1. *A*, Immunofluorescence studies of exogenes expressions (using anti-Flag antibody) and comparison with endogenous ones (using specific antibodies). *B*, Schematic representation of the two-step purification protocol.

Fig. S2. A, Relative amount of specific and non-specific peptides for each identified proteins (Dataset 1, sheets 1, 5, and 9) were plotted in function of their confident score. As shown for *A*, Ubiquitin, *B*, Nedd8, and *C*, SUMO1, the proportion of GFP background over samples became too important below the score of 50. Hence, only proteins identified with a score superior to 50 were considered as significant.

Fig. S3. *A*, Graph showing the repartition of indentified ubiquitylated proteins within biological processes (GO terms) in comparison with MiaPaCa2 proteome (Values > 1.5% were considered only). *B*, Graph

showing the repartition of indentified ubiquitylated proteins within Cell compartments (GO terms) in comparison with MiaPaCa2 proteome (Values > 1.5% were considered only).

Fig. S4. *A*, Repartition of indentified neddylated proteins within biological processes and, *B*, within cell compartments, in comparison with MiaPaCa2 proteome (Values > 1.5% were considered only).

Fig. S5. *A*, Repartition of indentified sumoylated proteins within biological processes and, *B*, within cell compartments categories, in comparison with MiaPaCa2 proteome (Values > 1.5% were considered only).

Fig. S6. Repartition of proteins modified by at least two modifiers (Ubiquitin + Nedd8; Ubiquitin + SUMO1; Nedd8 + SUMO1; Ubiquitin + Nedd8 + SUMO1) in normal conditions within *A*, biological processes and *B*, cell compartments categories.

Fig. S7. Repartition between positive and negative variations for proteins undergoing two different types of modifications following Gemcitabine treatment.

Fig. S8. *A*, Repartition of Gemcitabine induced altered ubiquitylations within biological processes and comparison with non-treated. *B*, Repartition of Gemcitabine induced altered neddylations within biological processes and comparison with non-treated. *C*, Repartition of Gemcitabine induced altered sumoylations within biological processes and comparison with non-treated.

Fig. S9. *A*, Differentially ubiquitylated proteins with known ligases (E3s) and/or deubiquitinase (DUBs) among their interacting partners. *B*, Differentially neddylated proteins with known interacting Nedd8 ligase (Nedd8 E3) and/or hydrolase (Nedd8 DUB), ubiquitin ligase and/or hydrolase, and SUMO ligase and/or hydrolase. *C*, Differentially sumoylated proteins with known SUMO ligase (SUMO E3) and/or

hydrolase (SUMO DUB), ubiquitin ligase and/or hydrolase, and Nedd8 ligase and/or hydrolases among their direct interactors.

Fig. S10. *A*, Gemcitabine induced variation of polyubiquitin chains types. *B*, Gemcitabine induced variation of polynedd8 chains types.

Fig. S11. *A*, SNIP1 sumoylation by SUMO2 has been studied by transfecting 293T cells with His6-SUMO2 in combination with GST-SNIP1 WT and mutants as indicated. Sumoylated proteins were isolated by Nickel pull down and SUMO2 modified SNIP1 revealed using anti-SNIP1 antibody. Expression of constructs in cell extract has been verified. *B*, MiaPaCa-2 6HF_SUMO1 cells were transfected with P38 alpha siRNA or control siRNA. 24h later, cells were treated or not with Gemcitabine as indicated. Sumoylated proteins were isolated by Nickel pull down and sumoylated SNIP1 revealed using anti-SNIP1 antibody. Proteins levels were verified in cell extract. *C*, 293T cells were transfected with GST-SNIP1, 6HF-SUMO1 and treated with the p38 inhibitor PD169316 or SB203580 as indicated. Sumoylated proteins were isolated by Nickel pull down and sumoylated SNIP1 revealed using anti-SNIP1 antibody. Expression of constructs in cell extract has been verified.

Fig. S12. Immunofluorescence study of SNIP1 intracellular localization in MiaPaCa2 cells in function of Gemcitabine treatment (10µM, 36h).

Datasets Legends

Dataset 1. Processing of data from LC-MS/MS. Sheet 1 "Ub": all identified proteins in ubiquitin screening with calculation of their confidence and scores. Sheet 2 "Ub >20": listing of ubiquitylated proteins with a confidence above 50. Sheet 3 "Ub >50 NT only: listing of ubiquitylated proteins in non treated cells only. Sheet 4 "Ub Gem +->50": Listing of proteins differentially ubiquitylated upon

Gemcitabine treatment with a variation score superior to +50 or inferior to -50. Sheets 5 to 8: same thing for Neddylated proteins. Sheets 9 to 12: same thing for sumoylated proteins.

Dataset 2. Comparison of Gemcitabine induced variations with those obtained by mass spectrometry peak quantification when it was possible.

Dataset 3. Lists of known interacting proteins for all differentially modified proteins upon Gemcitabine treatment, and lists of binaries associations within each group (Ubiquitin, Nedd8, SUMO1) (Fig. 4).

Dataset 4. Multiconsensus report for Ubiquitin samples

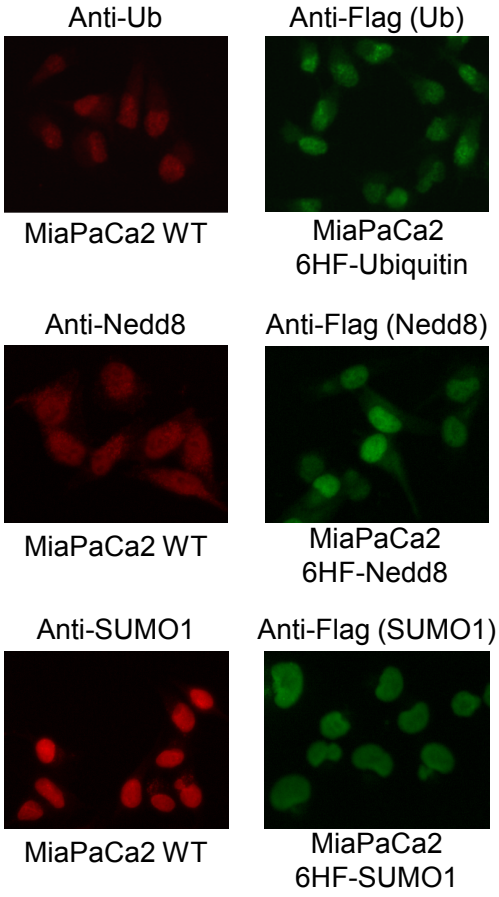
Dataset 5. Multiconsensus report for Nedd8 samples

Dataset 6. Multiconsensus report for SUMO1 samples

Dastaset 7. Report from iTRAQ study of MiaPaCa2 cells proteome in absence and presence of Gemcitabine.

Figure S1

A


Anti-Ub
Anti-Flag (Ub)
MiaPaCa2 WT
MiaPaCa2
6HF-Ubiquitin
Anti-Nedd8
Anti-Flag (Nedd8)
MiaPaCa2 WT
MiaPaCa2
6HF-Nedd8
Anti-SUMO1
Anti-Flag (SUMO1)
MiaPaCa2 WT
MiaPaCa2
6HF-SUMO1

B

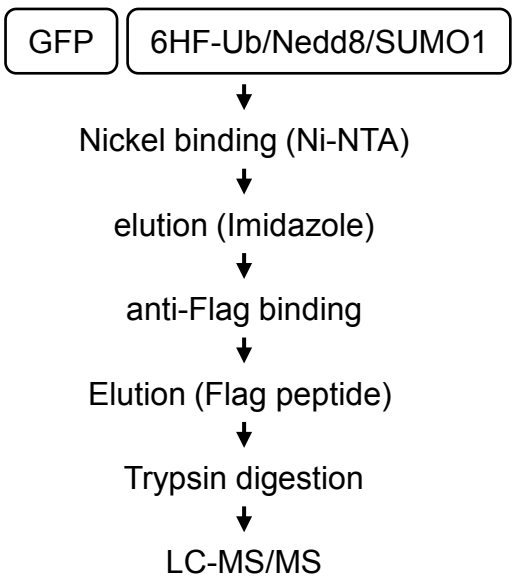

GFP
6HF-Ub/Nedd8/SUMO1
Nickel binding (Ni-NTA)
elution (Imidazole)
anti-Flag binding
Elution (Flag peptide)
Trypsin digestion
LC-MS/MS

Figure S2

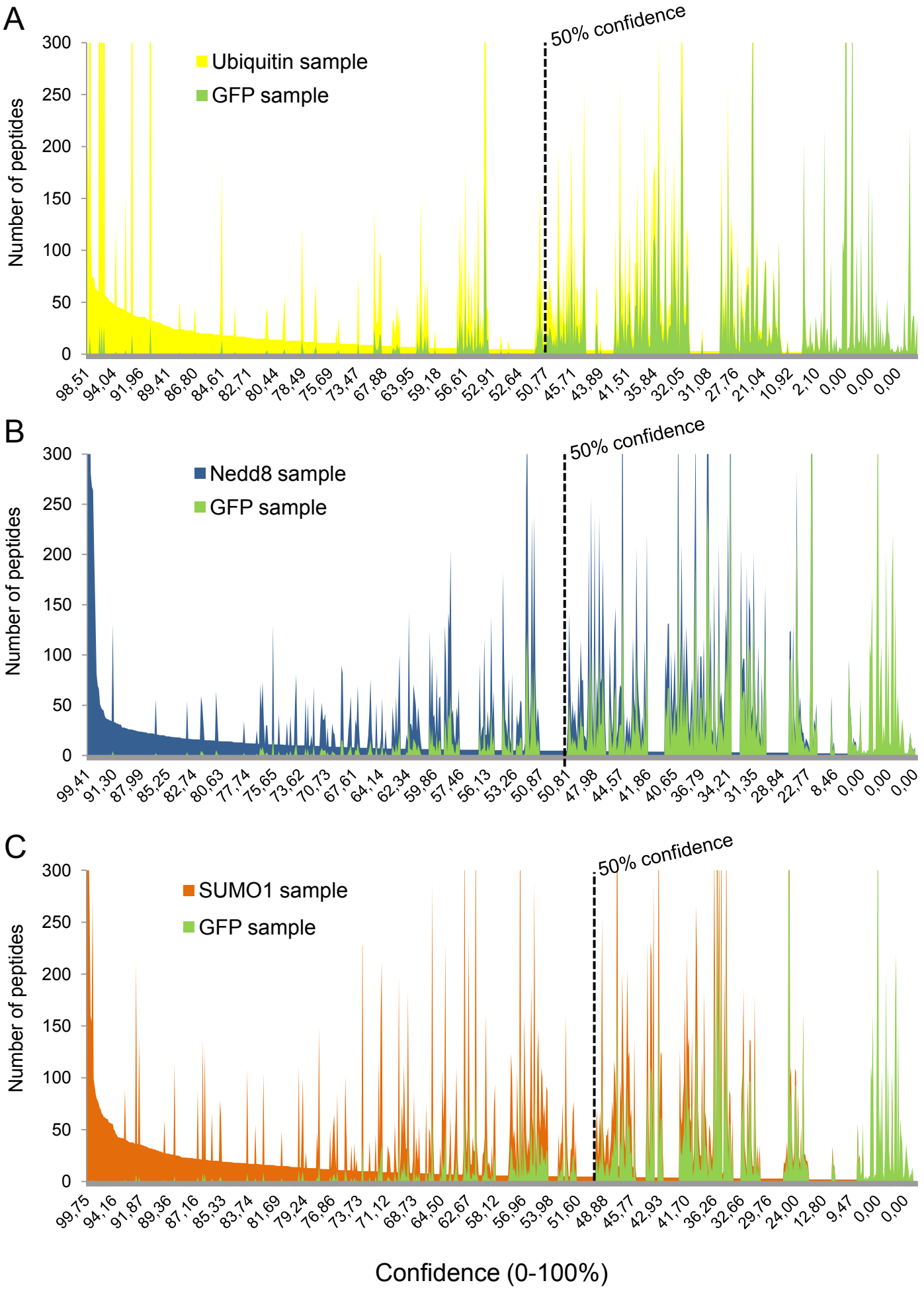

A
Ubiquitin sample
GFP sample
50% confidence
Number of peptides
300
250
200
150
100
50
0
98,51 94,04 91,96 89,41 86,80 84,61 82,71 80,44 78,49 75,69 73,47 67,88 63,95 59,18 56,61 52,91 52,64 50,77 45,71 43,89 41,51 35,84 32,05 31,08 27,76 21,04 10,92 2,10 0,00 0,00 0,00
B
Nedd8 sample
GFP sample
50% confidence
Number of peptides
300
250
200
150
100
50
0
99,41 91,30 87,99 85,25 82,74 80,63 77,74 75,65 73,62 70,73 67,61 64,14 62,34 59,86 57,46 56,13 53,26 50,87 50,81 47,98 44,57 41,86 40,65 36,79 34,21 31,35 28,84 22,77 8,46 0,00 0,00 0,00
C
SUMO1 sample
GFP sample
50% confidence
Number of peptides
300
250
200
150
100
50
0
99,75 94,16 91,87 89,36 87,16 85,33 83,74 81,69 79,24 76,86 73,73 71,12 68,73 64,50 62,67 58,12 56,96 53,98 51,60 48,88 45,77 42,93 41,70 36,26 32,66 29,76 24,00 12,80 9,47 0,00 0,00
Confidence (0-100%)

Figure S3

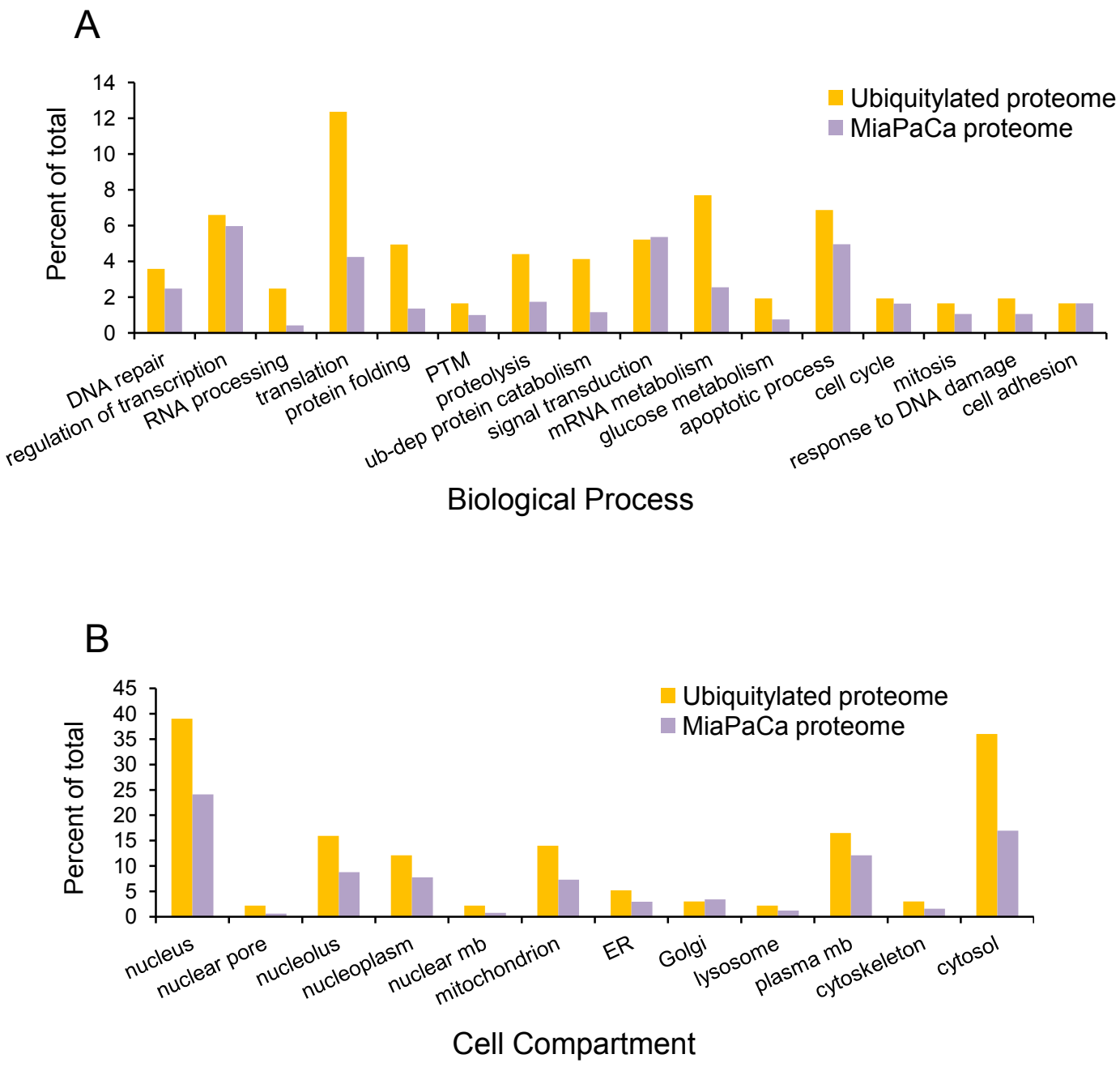

A
Percent of total
14
12
10
8
6
4
2
0
Ubiquitylated proteome
MiaPaCa proteome
DNA repair
regulation of transcription
RNA processing
translation
protein folding
PTM
proteolysis
ub-dep protein catabolism
signal transduction
mRNA metabolism
glucose metabolism
apoptotic process
cell cycle
mitosis
response to DNA damage
cell adhesion
Biological Process
B
Percent of total
45
40
35
30
25
20
15
10
5
0
Ubiquitylated proteome
MiaPaCa proteome
nucleus
nuclear pore
nucleolus
nucleoplasm
nuclear mb
mitochondrion
ER
Golgi
lysosome
plasma mb
cytoskeleton
cytosol
Cell Compartment

Figure S4

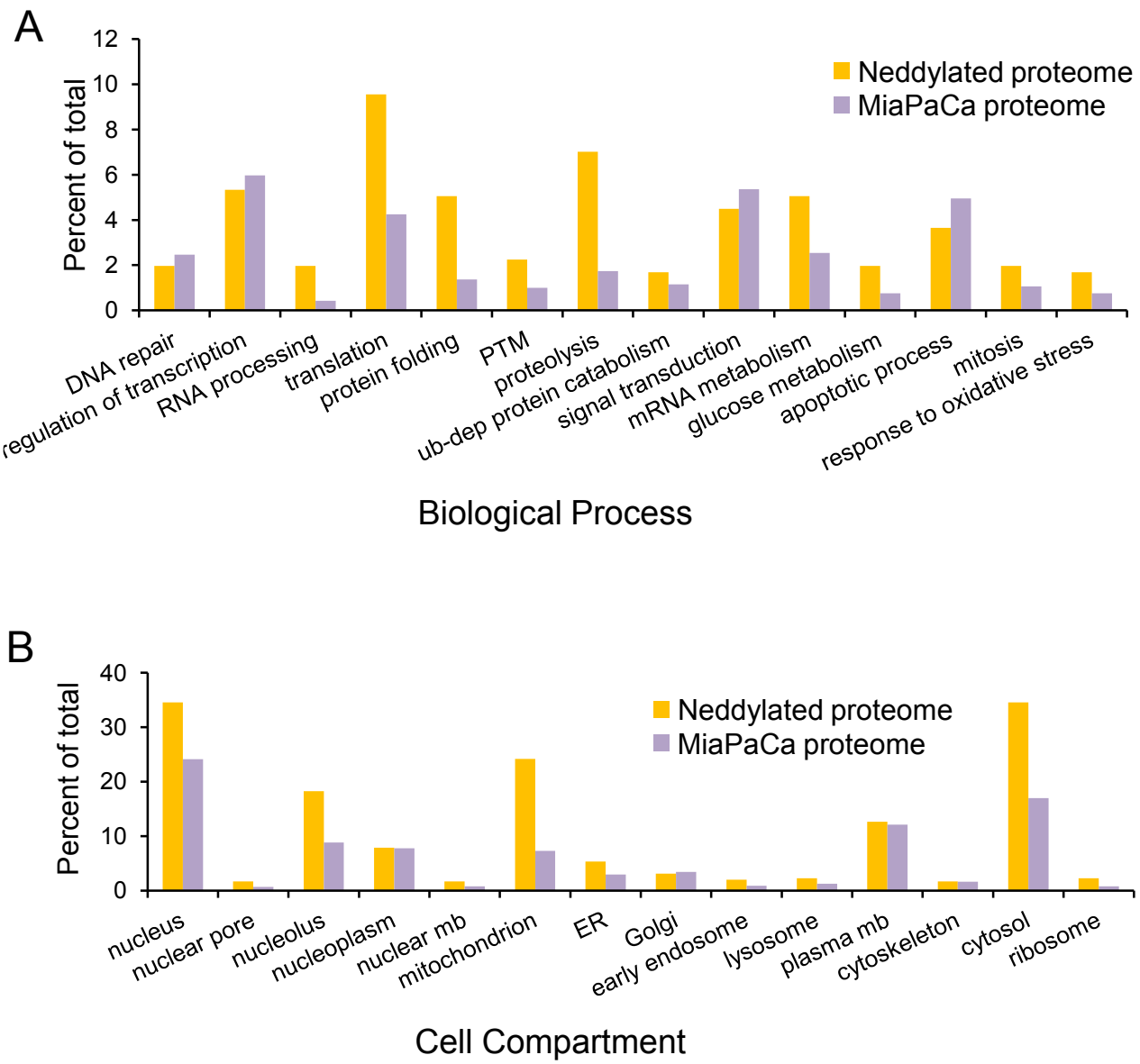

A
Percent of total
12
10
8
6
4
2
0
Neddylated proteome
MiaPaCa proteome
DNA repair
regulation of transcription
RNA processing
translation
protein folding
PTM
proteolysis
ub-dep protein catabolism
signal transduction
mRNA metabolism
glucose metabolism
apoptotic process
mitosis
response to oxidative stress
Biological Process
B
Percent of total
40
30
20
10
0
Neddylated proteome
MiaPaCa proteome
nucleus
nuclear pore
nucleolus
nucleoplasm
nuclear mb
mitochondrion
ER
Golgi
early endosome
lysosome
plasma mb
cytoskeleton
cytosol
ribosome
Cell Compartment

Figure S5

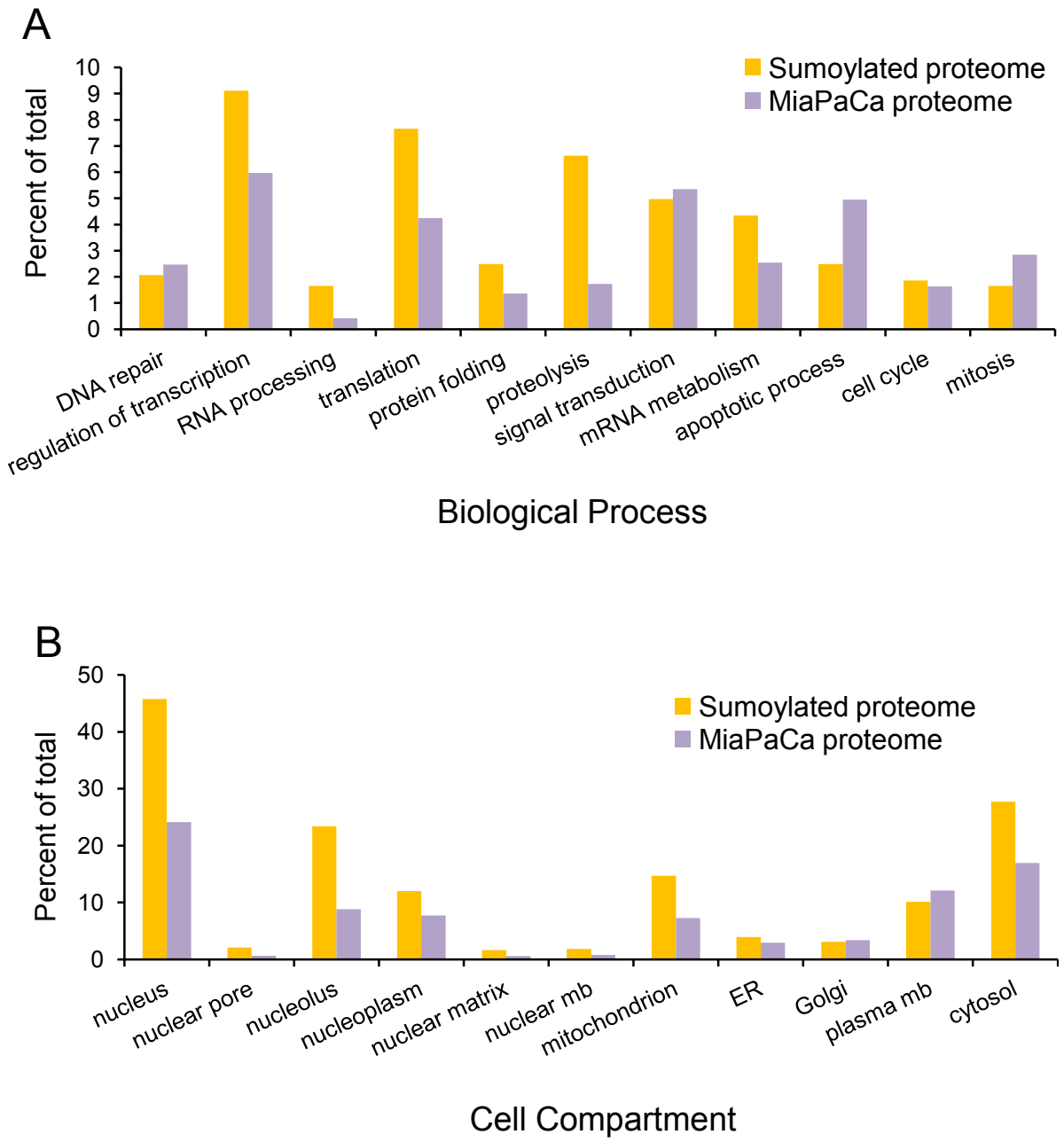

A
Percent of total
10
9
8
7
6
5
4
3
2
1
0
Sumoylated proteome
MiaPaCa proteome
DNA repair
regulation of transcription
RNA processing
translation
protein folding
proteolysis
signal transduction
mRNA metabolism
apoptotic process
cell cycle
mitosis
Biological Process
B
Percent of total
50
40
30
20
10
0
Sumoylated proteome
MiaPaCa proteome
nucleus
nuclear pore
nucleolus
nucleoplasm
nuclear matrix
nuclear mb
mitochondrion
ER
Golgi
plasma mb
cytosol
Cell Compartment

Figure S6

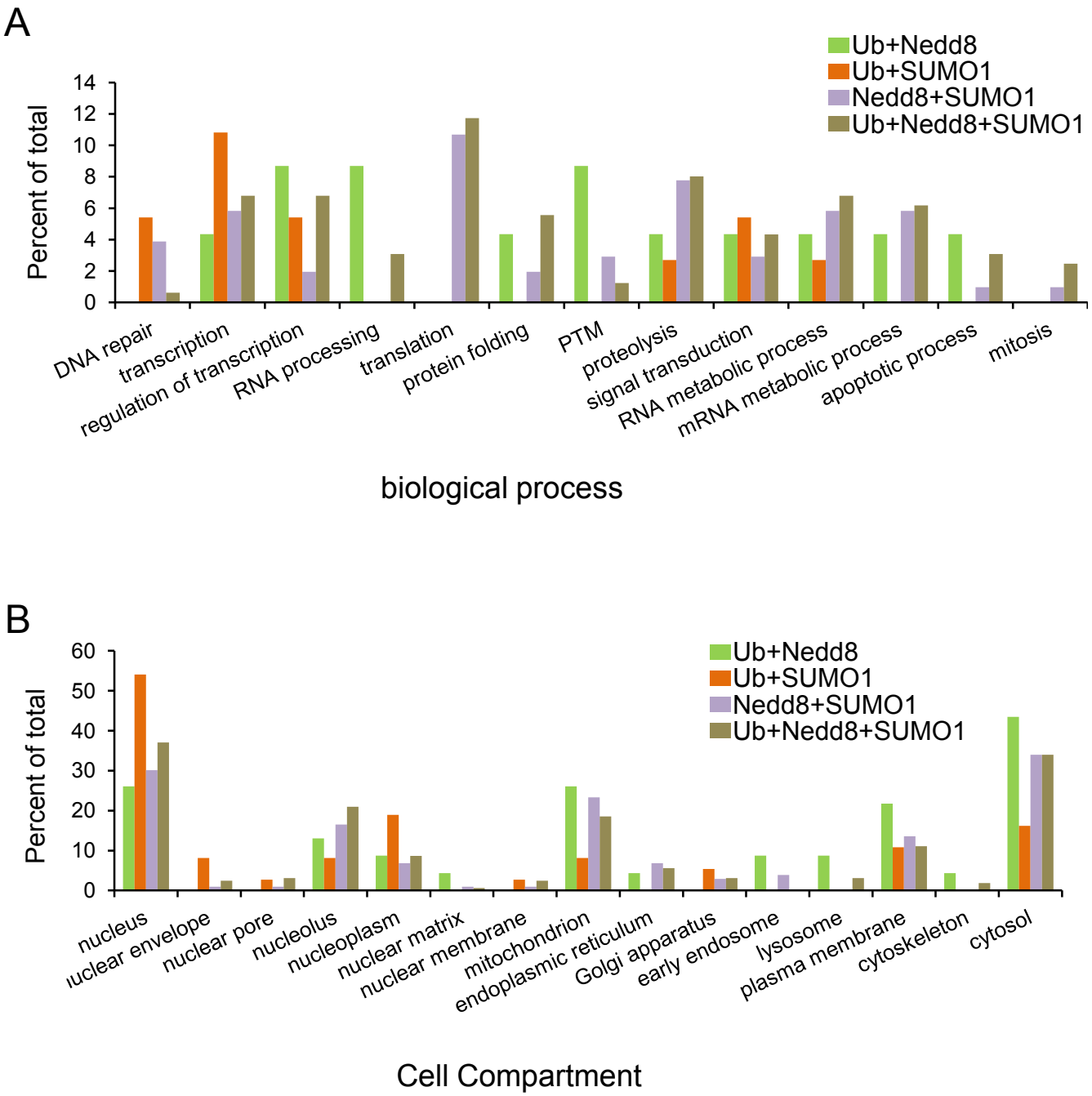

A
Ub+Nedd8
Ub+SUMO1
Nedd8+SUMO1
Ub+Nedd8+SUMO1
Percent of total
0
2
4
6
8
10
12
14
DNA repair
transcription
regulation of transcription
RNA processing
translation
protein folding
PTM
proteolysis
signal transduction
RNA metabolic process
mRNA metabolic process
apoptotic process
mitosis
biological process
B
Ub+Nedd8
Ub+SUMO1
Nedd8+SUMO1
Ub+Nedd8+SUMO1
Percent of total
0
10
20
30
40
50
60
nucleus
nuclear envelope
nuclear pore
nucleolus
nucleoplasm
nuclear matrix
nuclear membrane
mitochondrion
endoplasmic reticulum
Golgi apparatus
early endosome
lysosome
plasma membrane
cytoskeleton
cytosol
Cell Compartment

Figure S7

Variation + or -	+/+	+/-	-/+	-/-	Total
Ub/Nedd8	4	2	4	0	10
Ub/SUMO1	2	4	1	1	8
Nedd8/SUMO1	5	6	0	3	14
Total	11	12	5	4	

Figure S8

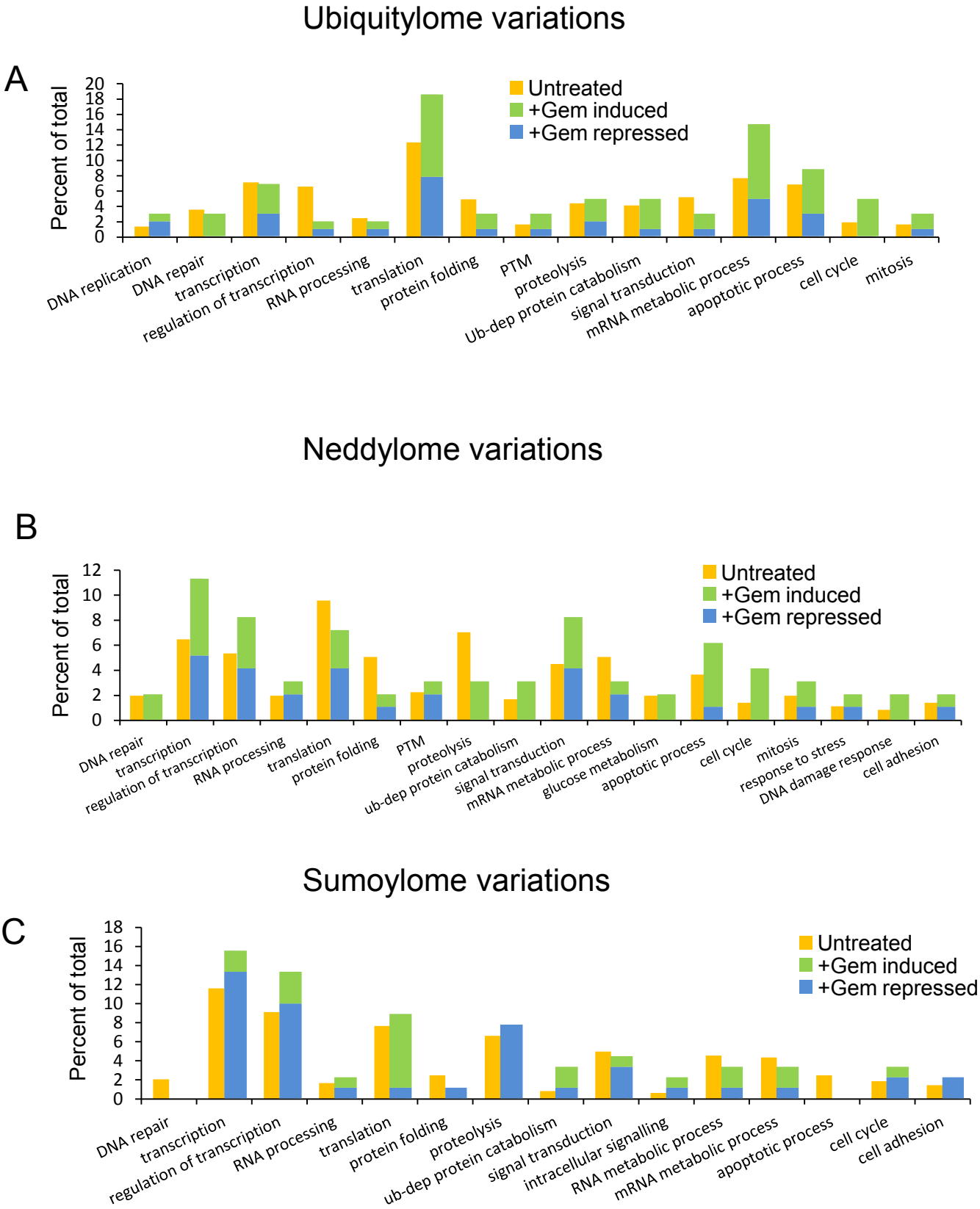

Ubiquitylome variations
A
Percent of total
0
2
4
6
8
10
12
14
16
18
20
Untreated
+Gem induced
+Gem repressed
DNA replication
DNA repair
transcription
regulation of transcription
RNA processing
translation
protein folding
PTM
proteolysis
Ub-dep protein catabolism
signal transduction
mRNA metabolic process
apoptotic process
cell cycle
mitosis
Neddylome variations
B
Percent of total
0
2
4
6
8
10
12
Untreated
+Gem induced
+Gem repressed
DNA repair
transcription
regulation of transcription
RNA processing
translation
protein folding
PTM
proteolysis
ub-dep protein catabolism
signal transduction
mRNA metabolic process
glucose metabolism
apoptotic process
cell cycle
mitosis
response to stress
DNA damage response
cell adhesion
Sumoylome variations
C
Percent of total
0
2
4
6
8
10
12
14
16
18
Untreated
+Gem induced
+Gem repressed
DNA repair
transcription
regulation of transcription
RNA processing
translation
protein folding
proteolysis
ub-dep protein catabolism
signal transduction
intracellular signalling
RNA metabolic process
mRNA metabolic process
apoptotic process
cell cycle
cell adhesion

Figure S9

A Know ligases and DUBs among interactors for ubiquitin variation

DUBs \ E3s	0	1	2	3	4	5	7	14
0	56	17	4	3	-	1	-	-
1	4	4	1	1	1	-	-	-
2	1	1	-	-	-	-	-	-
3	-	1	-	1	-	-	-	-
4	1	3	-	-	-	-	1	1

B Know ligases and DUBs among interactors for Nedd8 variation

	Nedd8 E3	Nedd8 DUB	Ub E3	Ub DUB	SUMO E3	SUMO DUB
RPL11	-	1 (SENP8)	2	-	-	-
HSPA5	-	1 (COPS5)	3	1	-	-
TP53	-	1 (COPS5)	18	6	4	-

C Know ligases and DUBs among interactors for SUMO1 variation

	SUMO E3	SUMO DUB	Ub E3	Ub DUB	Nedd8 E3	Nedd8 DUB
SNIP1	4 (PIAS1,2,4, TOPORS)	-	1	-	-	-
TRIM24	1 (TRIM28)	-	3	-	-	-
PIAS2	1 (PIAS1)	-	-	-	-	-

Figure S10

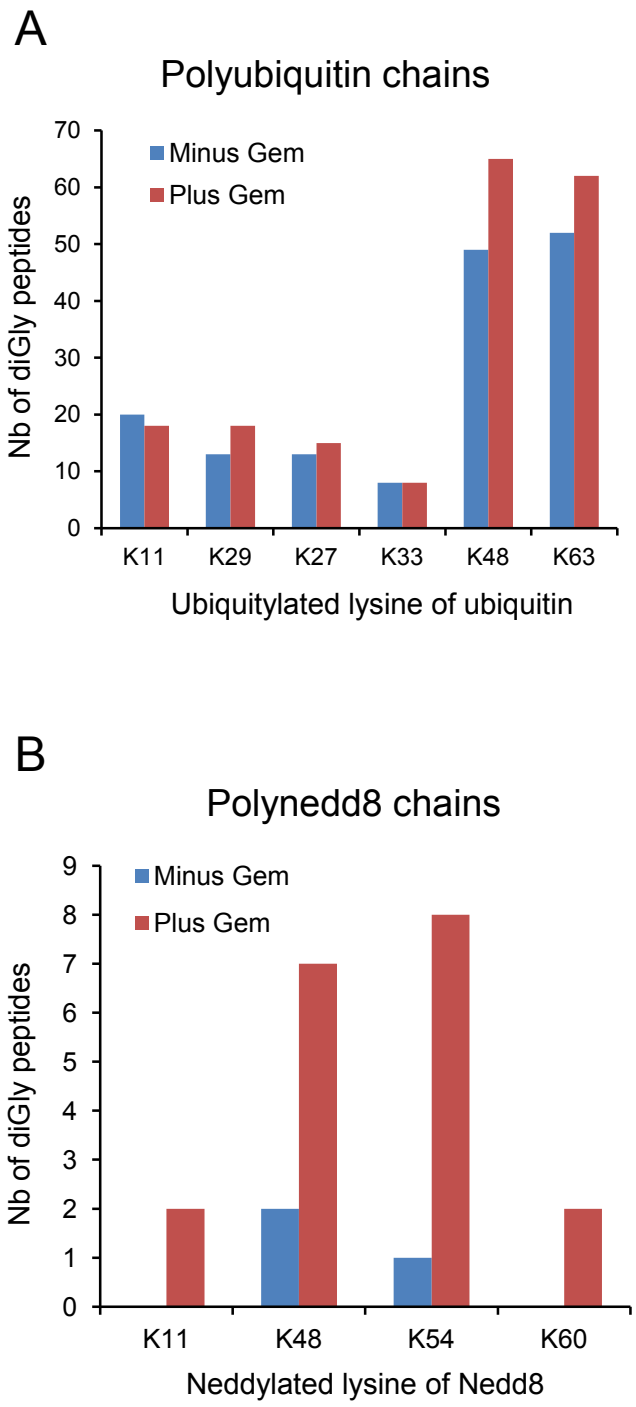
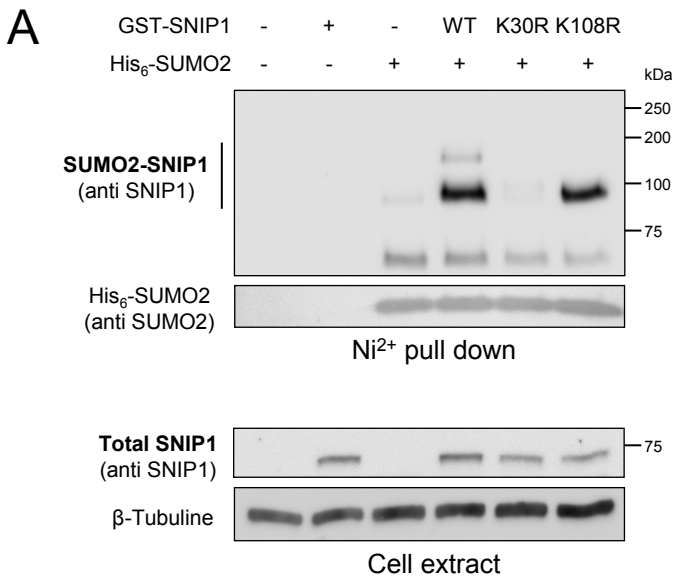
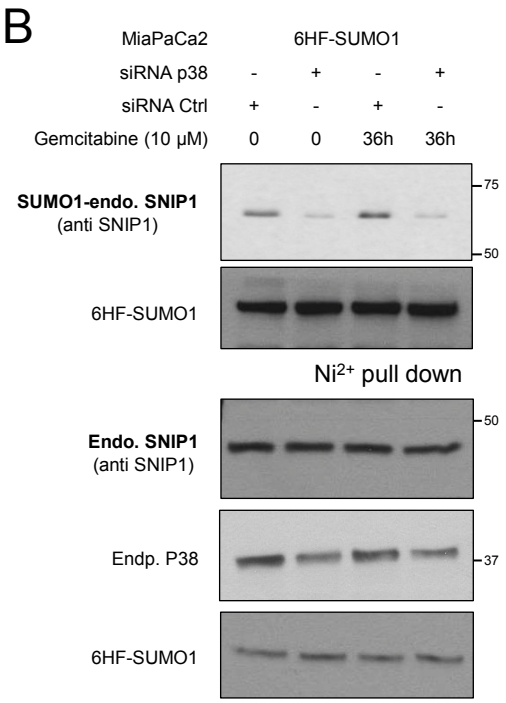
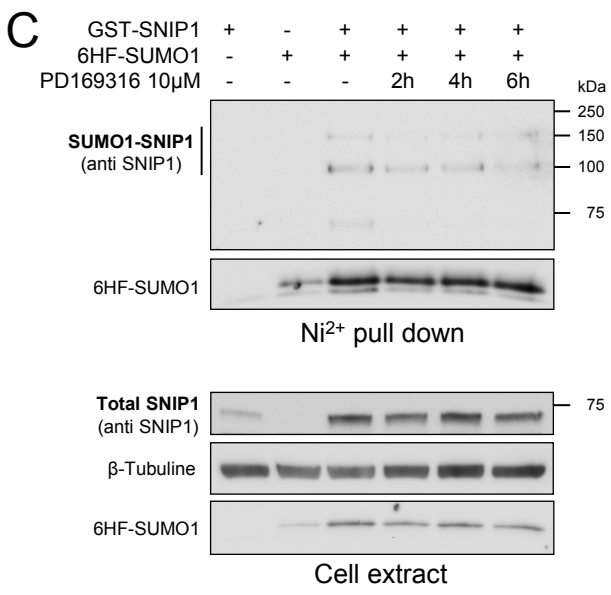

A
Polyubiquitin chains
Minus Gem
Plus Gem
Nb of diGly peptides
0
10
20
30
40
50
60
70
K11
K29
K27
K33
K48
K63
Ubiquitylated lysine of ubiquitin
B
Polynedd8 chains
Minus Gem
Plus Gem
Nb of diGly peptides
0
1
2
3
4
5
6
7
8
9
K11
K48
K54
K60
Neddylated lysine of Nedd8

Figure S11


A
GST-SNIP1 - + - WT K30R K108R
His6-SUMO2 - - + + + +
kDa
250
200
100
75
SUMO2-SNIP1
(anti SNIP1)
His6-SUMO2
(anti SUMO2)
Ni2+ pull down
Total SNIP1
(anti SNIP1)
β-Tubuline
75
Cell extract


B
MiaPaCa2 6HF-SUMO1
siRNA p38 - + - +
siRNA Ctrl + - + -
Gemcitabine (10 μM) 0 0 36h 36h
SUMO1-endo. SNIP1
(anti SNIP1)
75
50
6HF-SUMO1
Ni2+ pull down
Endo. SNIP1
(anti SNIP1)
50
Endp. P38
37
6HF-SUMO1


C
GST-SNIP1 + - + + + +
6HF-SUMO1 - + + + + +
PD169316 10μM - - - 2h 4h 6h
kDa
250
150
100
75
SUMO1-SNIP1
(anti SNIP1)
6HF-SUMO1
Ni2+ pull down
Total SNIP1
(anti SNIP1)
β-Tubuline
6HF-SUMO1
75
Cell extract

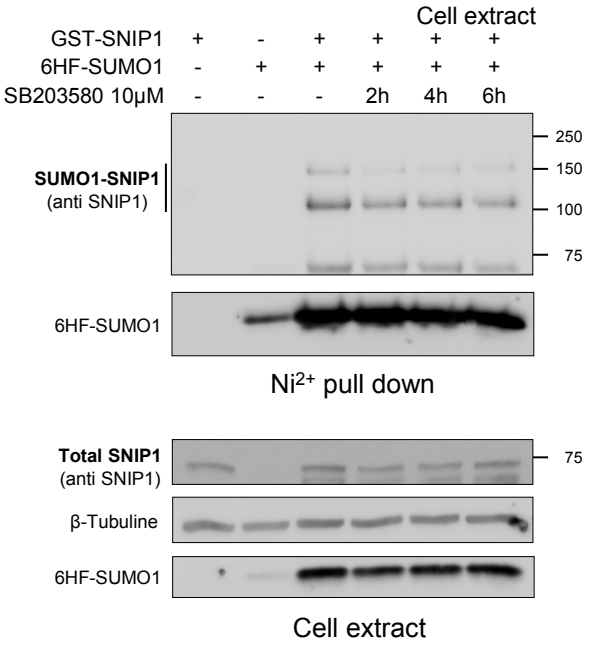
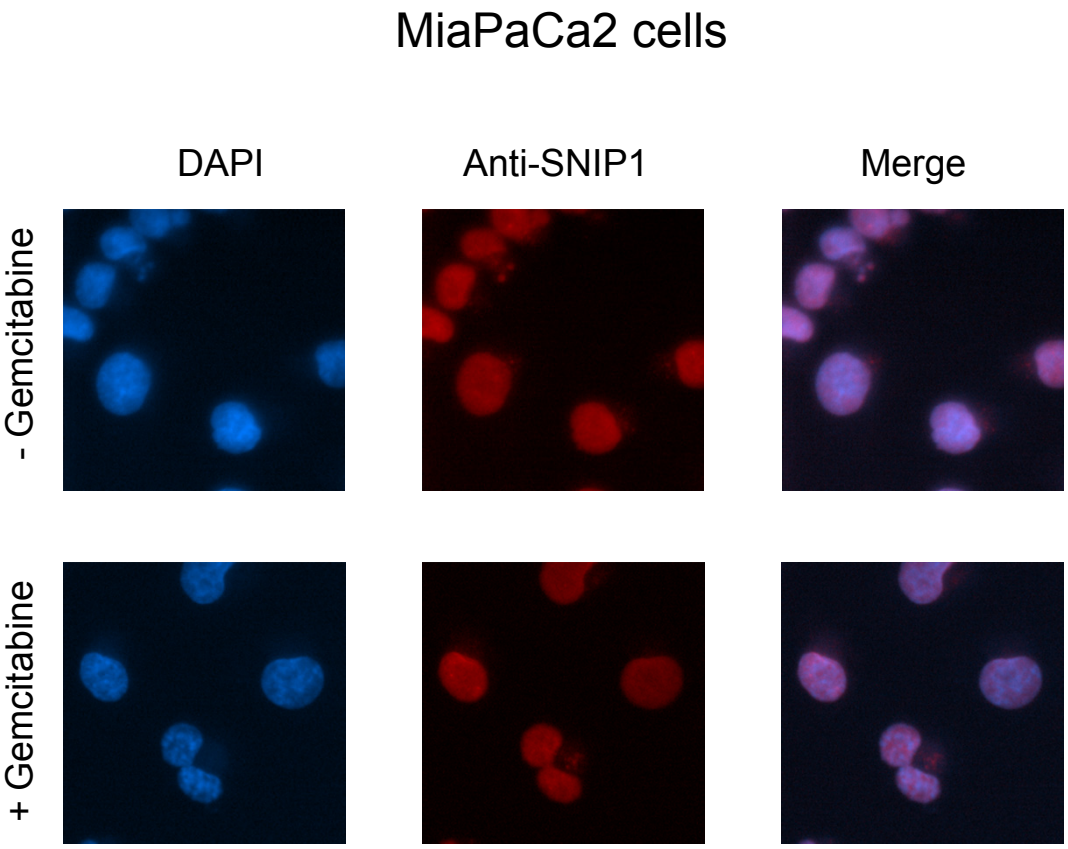

Cell extract
GST-SNIP1 + - + + + +
6HF-SUMO1 - + + + + +
SB203580 10μM - - - 2h 4h 6h
250
150
100
75
SUMO1-SNIP1
(anti SNIP1)
6HF-SUMO1
Ni2+ pull down
Total SNIP1
(anti SNIP1)
β-Tubuline
6HF-SUMO1
75
Cell extract

Figure S12


MiaPaCa2 cells
DAPI
Anti-SNIP1
Merge
- Gemcitabine
+ Gemcitabine