Figure S3

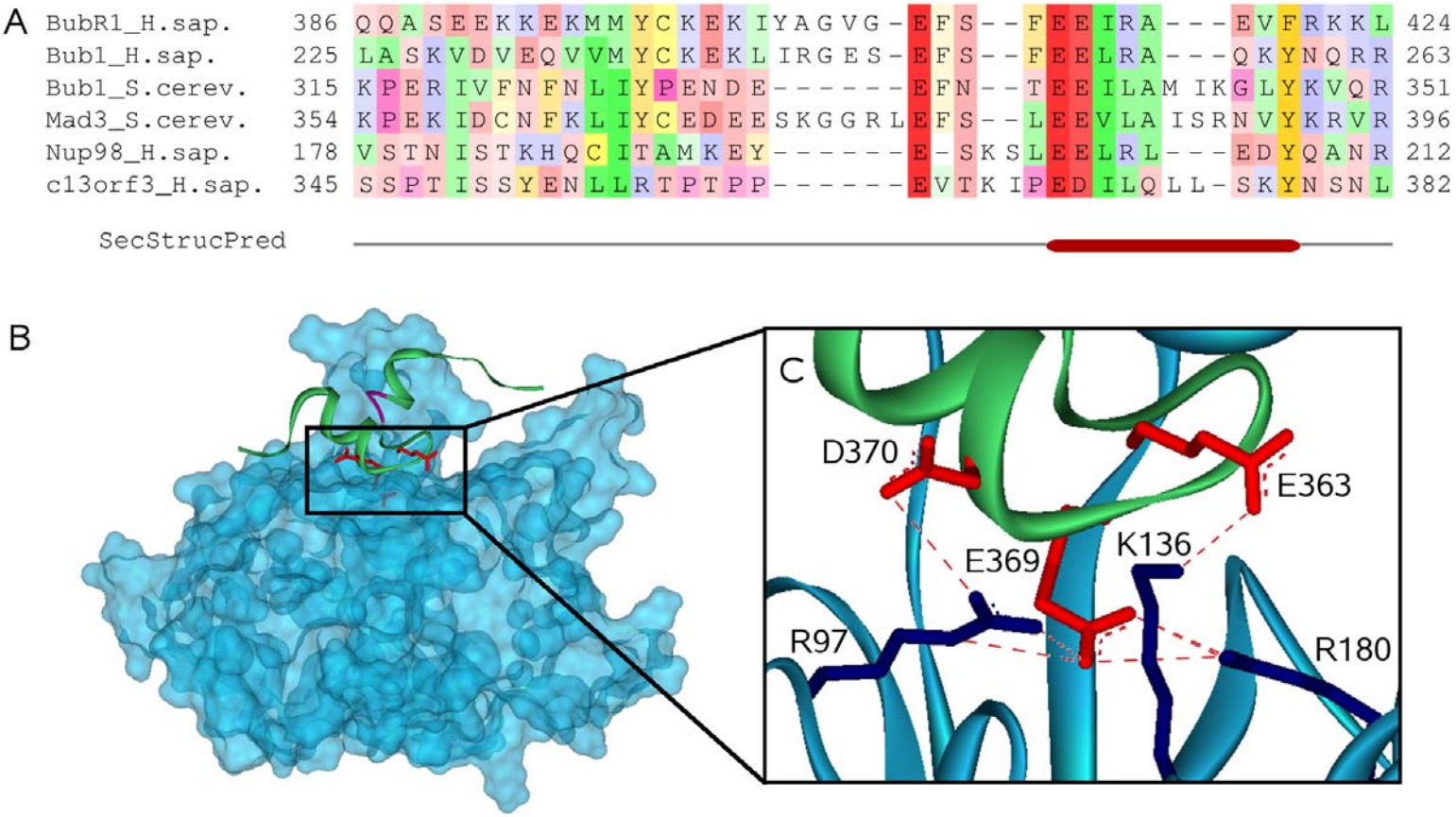
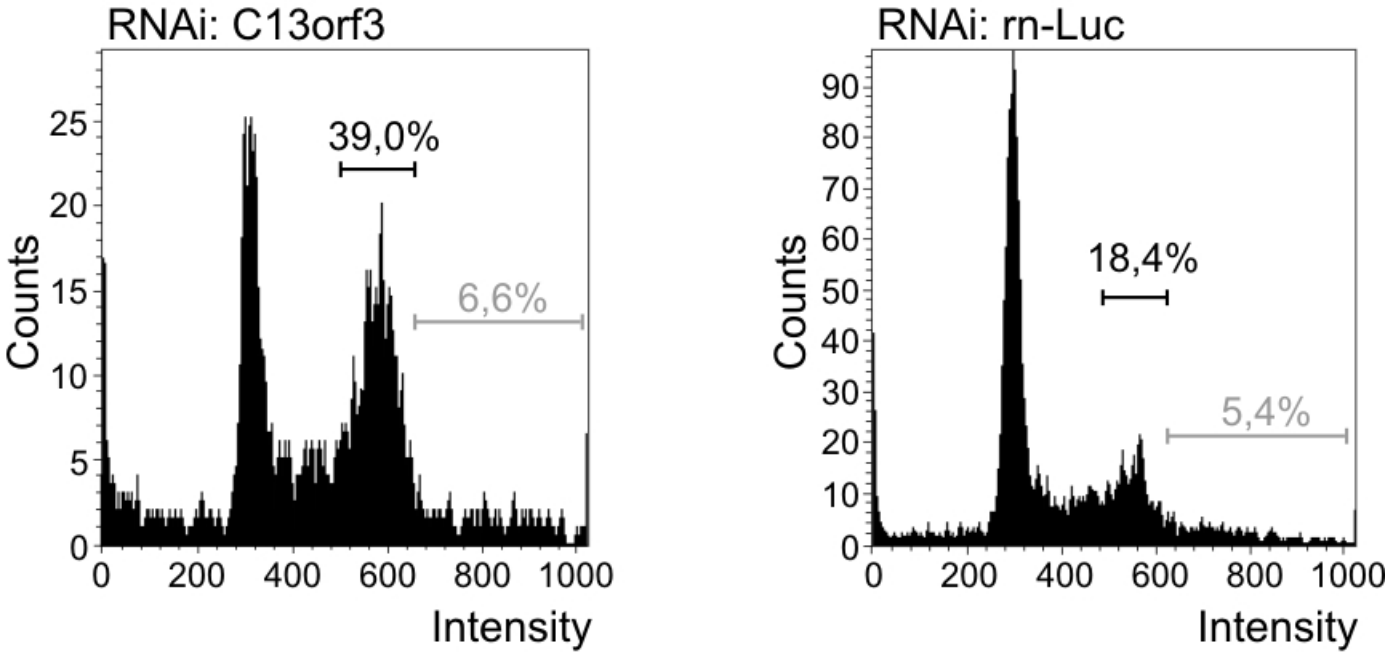

A
BubR1_H.sap. 386 QQASEEKKEKMMYCKEKIYAGVG-EFS--FEEIRA---EVFRKKL 424
Bub1_H.sap. 225 LASKVDVEQVVMYCKEKLIRGES-EFS--FEELRA---QKYNQRR 263
Bub1_S.cerev. 315 KPERIVFNFNLIYPENDE-------EFN--TEEILAMIKGLYKVQR 351
Mad3_S.cerev. 354 KPEKIDCNFKLIYCEDEESKGGRLEFS--LEEVLAISRNVYKRVR 396
Nup98_H.sap. 178 VSTNISTKHQCITAMKEY-------E-SKSLEELRL---EDYQANR 212
c13orf3_H.sap. 345 SSPTISSYENLLRTPTPP-------EVTKIPEDILQLL-SKYNSNL 382
SecStrucPred
B
C
D370
E363
E369
K136
R97
R180

Figure S6

A


RNAi: C13orf3
39,0%
6,6%
Counts
Intensity
RNAi: rn-Luc
18,4%
5,4%
Counts
Intensity

B

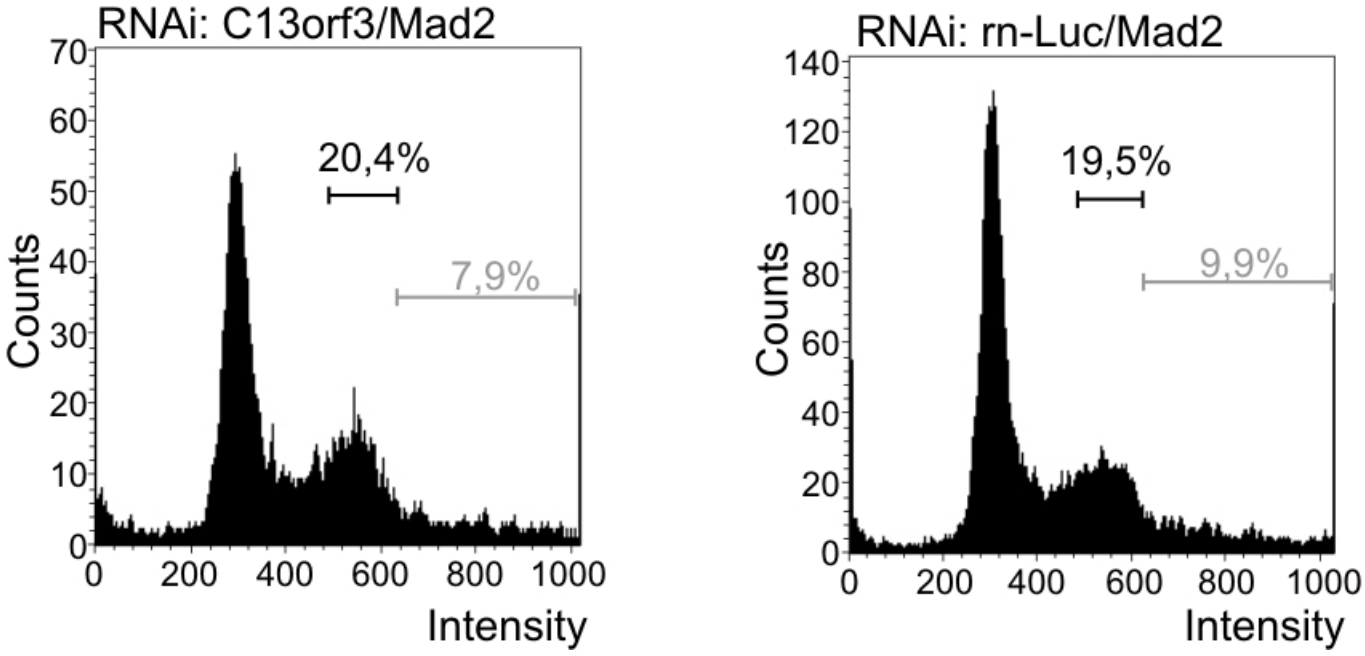
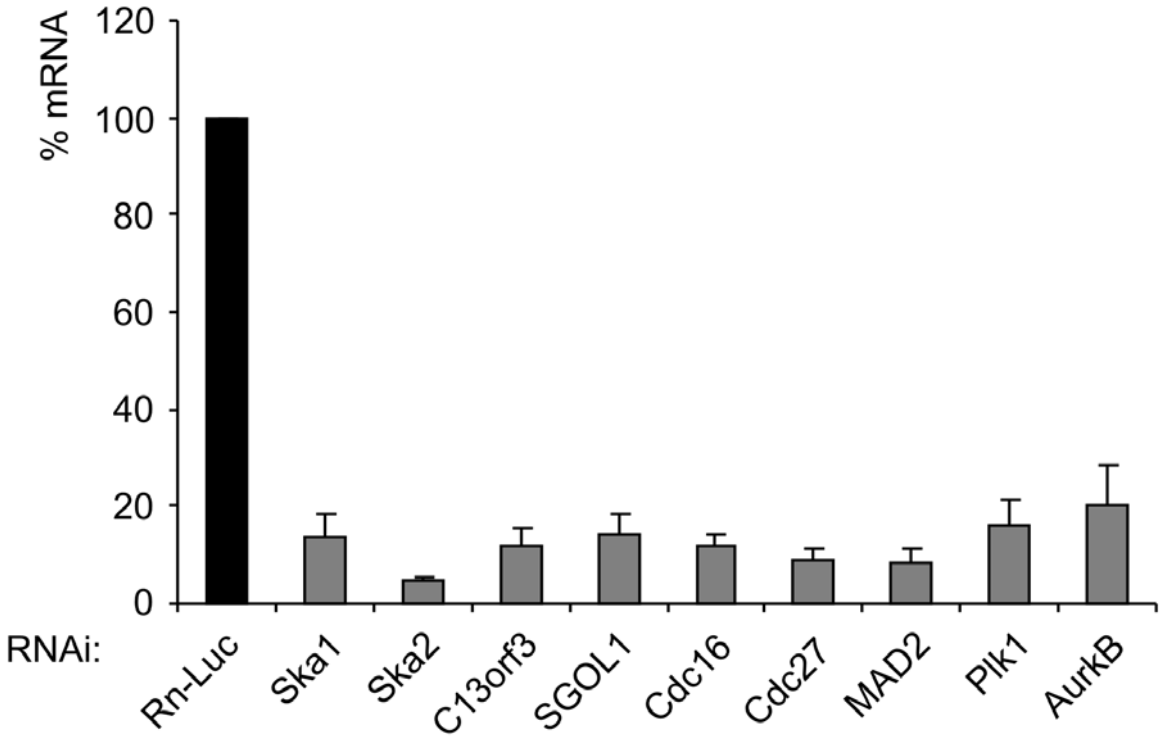
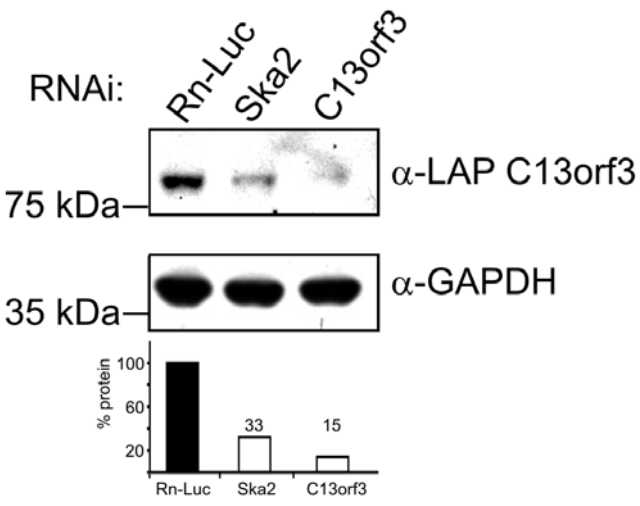

RNAi: C13orf3/Mad2
20,4%
7,9%
Counts
Intensity
RNAi: rn-Luc/Mad2
19,5%
9,9%
Counts
Intensity

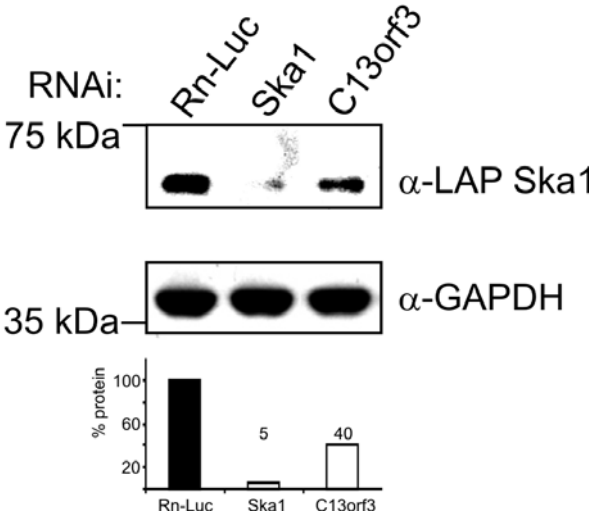
Figure S7

A


% mRNA
120
100
80
60
40
20
0
RNAi:
Rn-Luc
Ska1
Ska2
C13orf3
SGOL1
Cdc16
Cdc27
MAD2
Plk1
AurkB

B


RNAi:
Rn-Luc
Ska2
C13orf3
75 kDa
α-LAP C13orf3
35 kDa
α-GAPDH
% protein
100
60
20
33
15
Rn-Luc
Ska2
C13orf3


RNAi:
Rn-Luc
Ska1
C13orf3
75 kDa
α-LAP Ska1
35 kDa
α-GAPDH
% protein
100
60
20
5
40
Rn-Luc
Ska1
C13orf3

Supplementary information S8

Q-PCR primer sequences:

Ska1 – C18orf24 (ENSG00000154839):
LEFT PRIMER CGAAGGATACCAAAGGTCGT
RIGHT PRIMER CTCCGGCAGTGTCGTAAAAT

Ska2 – Fam33a (ENSG00000182628):
LEFT PRIMER AGAGCCGCATTTGTGCTACT
RIGHT PRIMER TGCCGCAGTTTTCTCTTCTT

C13orf3 (ENSG00000165480):
LEFT PRIMER TCCTGGTTTGAAAATTCCATCT
RIGHT PRIMER TCGTAGGTGAAGAGGGATCTG

SGOL1 (ENSG00000129810):
LEFT PRIMER TCTGGAATGGACCCCAATAG
RIGHT PRIMER TCTCCTTGTCCTGGAAGTTCA

MAD2 (ENSG00000164109):
LEFT PRIMER CTTCTCATTCGGCATCAACA
RIGHT PRIMER GAGTCCGTATTTCTGCACTCG

Cdc16 (ENSG00000130177):
LEFT PRIMER AGGGACGCTTGTAGAGCTGA
RIGHT PRIMER ATCCCACTGCAAACCAAGAC

Cdc27 (ENSG00000004897):
LEFT PRIMER GCAAATTTCACAGAGCCTCA
RIGHT PRIMER ACGAGGGATTCTTTGGGAAC

Plk1 (ENSG00000166851) :
LEFT PRIMER CATCCTGCACCTCAGCAAC
RIGHT PRIMER CATCAGTGGGCACAAGATGA

AurkB (ENSG00000178999):
LEFT PRIMER GGGAGAGCTGAAGATTGCTG
RIGHT PRIMER GCACCACAGATCCACCTTCT

Figure S12

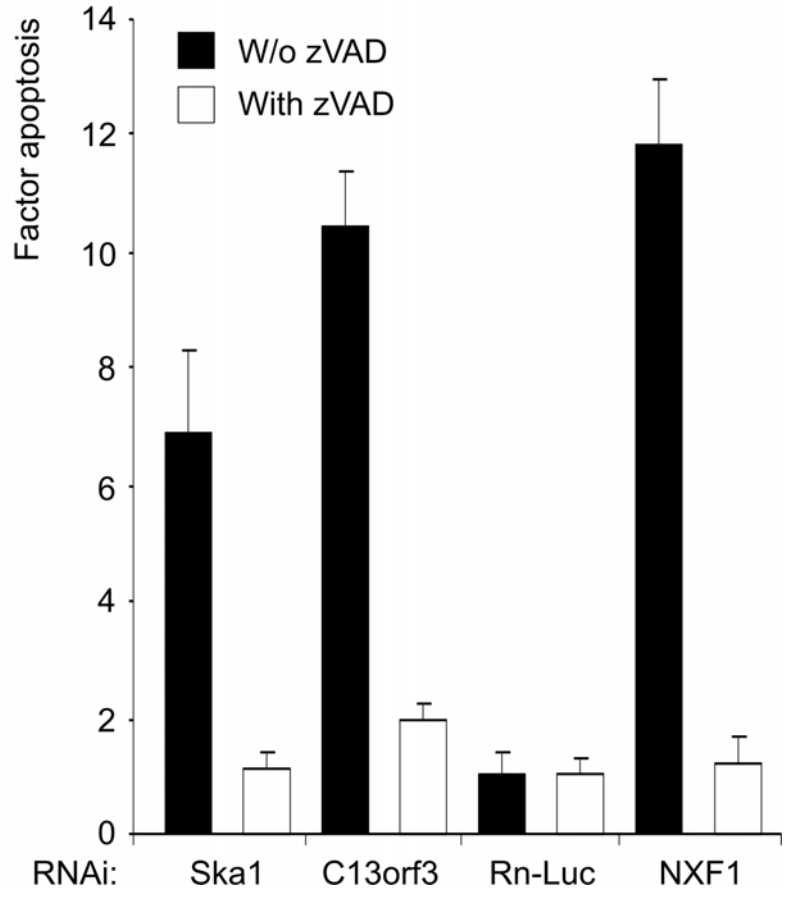
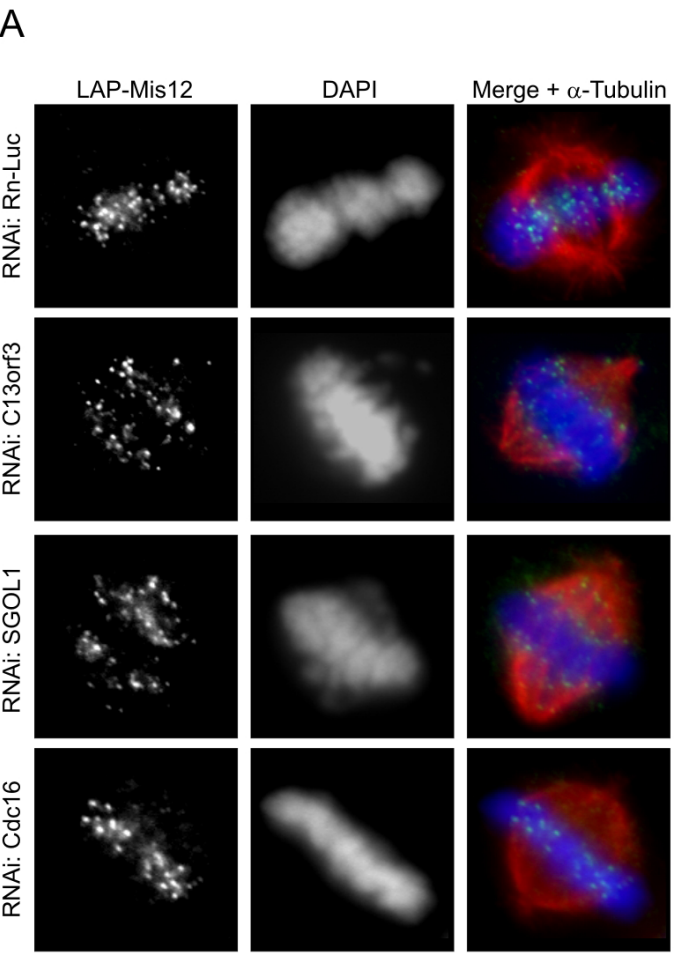
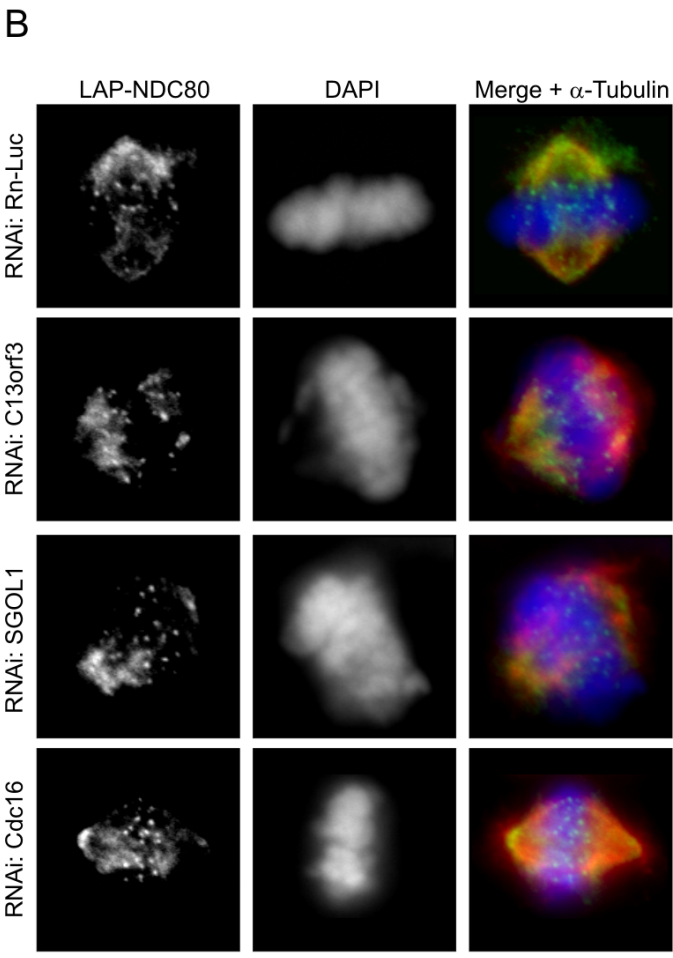

Factor apoptosis
14
12
10
8
6
4
2
0
W/o zVAD
With zVAD
RNAi:
Ska1
C13orf3
Rn-Luc
NXF1

Figure S13


A
LAP-Mis12
DAPI
Merge + α-Tubulin
RNAi: Rn-Luc
RNAi: C13orf3
RNAi: SGOL1
RNAi: Cdc16


B
LAP-NDC80
DAPI
Merge + α-Tubulin
RNAi: Rn-Luc
RNAi: C13orf3
RNAi: SGOL1
RNAi: Cdc16

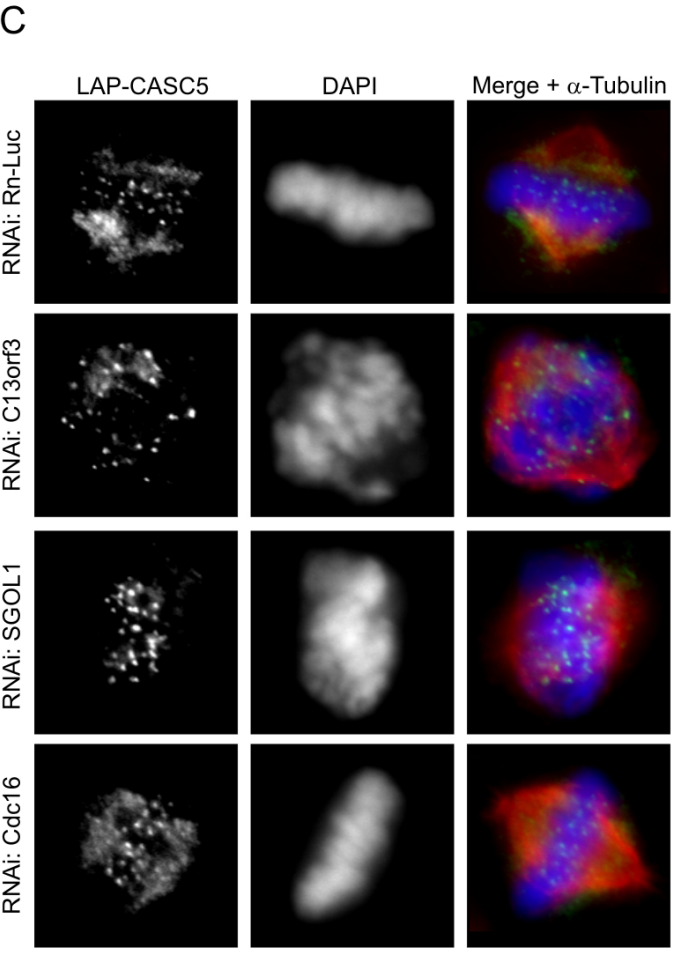
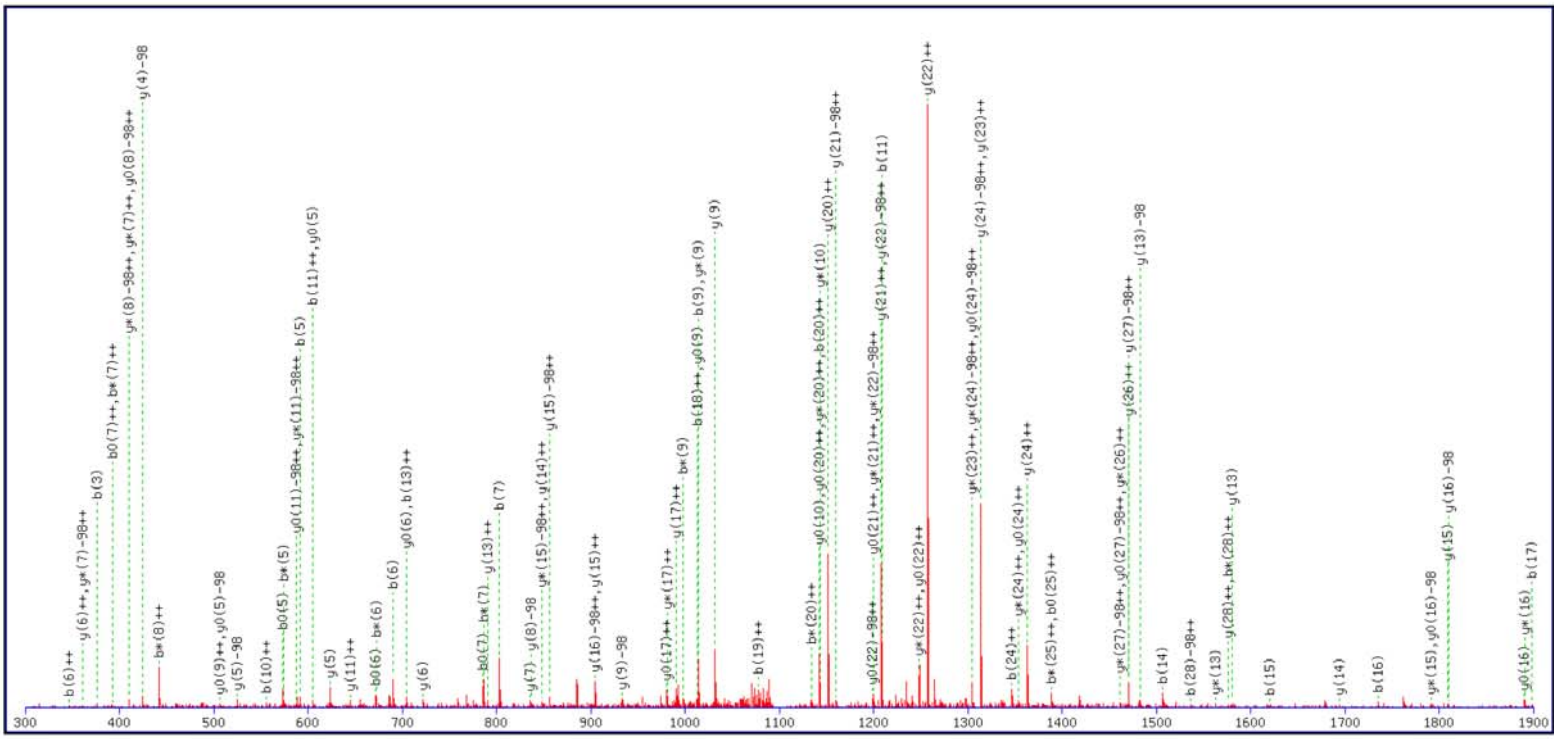

C
LAP-CASC5
DAPI
Merge + α-Tubulin
RNAi: Rn-Luc
RNAi: C13orf3
RNAi: SGOL1
RNAi: Cdc16

Figure S24

A

MS/MS Fragmentation of YIVSQVLPNPPQAVNNYKEEPVIVTPPTK

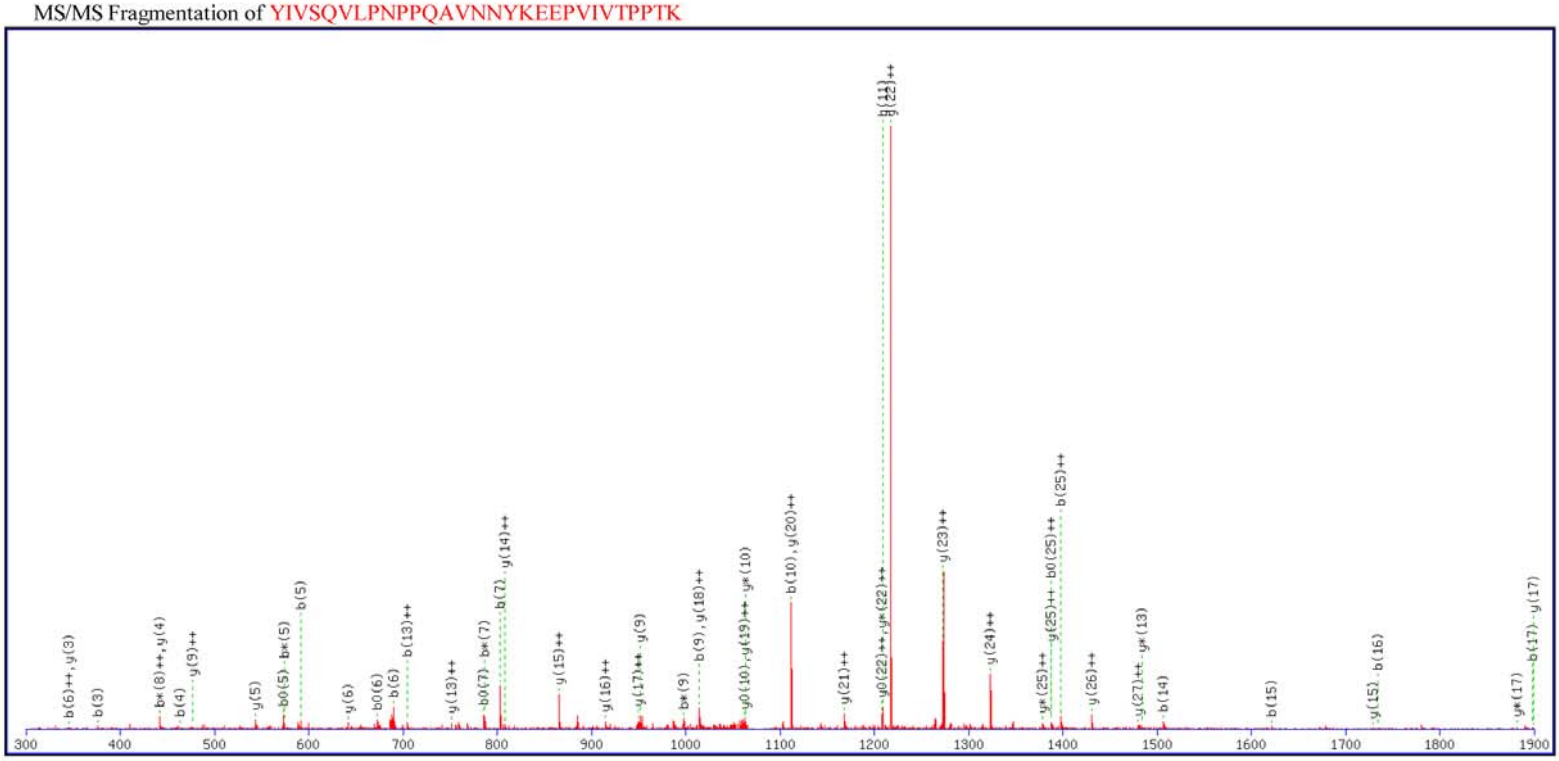


Monoisotopic mass of neutral peptide Mr(calc): 3313.6999

Ions Score: 41 **Expect:** 0.0011

B

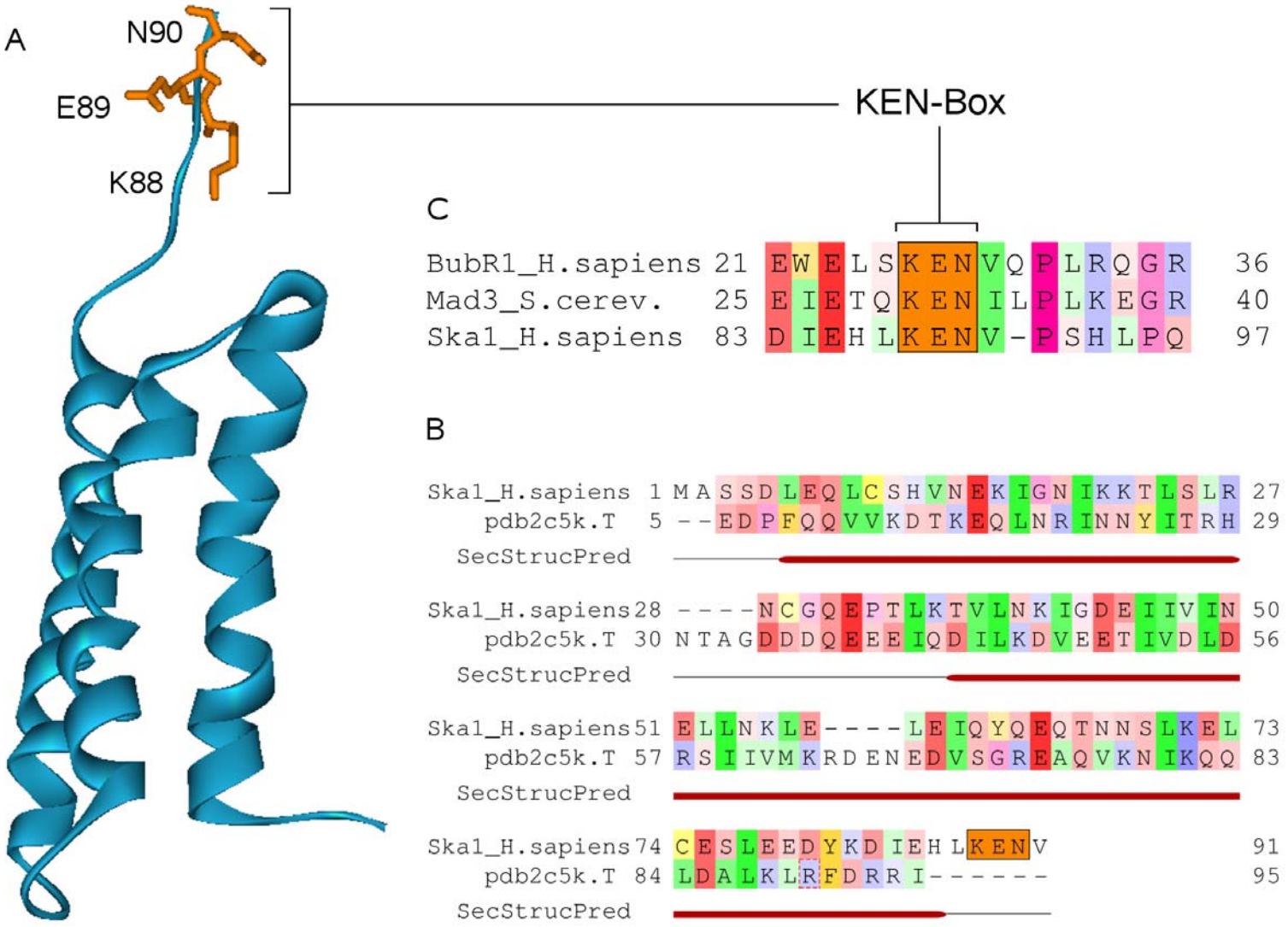
MS/MS Fragmentation of YIVSQVLPNPPQAVNNYKEEPVIVTPPTK



Monoisotopic mass of neutral peptide Mr(calc): 3233.7336

Ions Score: 51 **Expect:** 4.2e-05

Figure S25


A
N90
E89
K88
KEN-Box
C
BubR1_H.sapiens 21 EWELSKENVQPLRQGR 36
Mad3_S.cerev. 25 EIETQKENILPLKEGR 40
Ska1_H.sapiens 83 DIEHLKENV-PSHLPQ 97
B
Ska1_H.sapiens 1 MASSDLEQLCSHVNEKIGNIKKTLSLR 27
pdb2c5k.T 5 --EDPFQQVVKDTKEQLNRINNYITRH 29
SecStrucPred
Ska1_H.sapiens 28 ----NCGQEPTLKTVLNKIGDEIIVIN 50
pdb2c5k.T 30 NTAGDDDQEEEIQDILKDVEETIVDLD 56
SecStrucPred
Ska1_H.sapiens 51 ELLNKLE----LEIQYQEQTNNSLKEL 73
pdb2c5k.T 57 RSIIVMKRDENEDVSGREAQVKNIKQQ 83
SecStrucPred
Ska1_H.sapiens 74 CESLEEDYKDIEHLKENV 91
pdb2c5k.T 84 LDALKLRFDRRI------ 95
SecStrucPred

Supplementary information S26

Mass spectrometry

For assays performed using PPP2R2B as bait the cysteine bonds were reduced with 5 mM TCEP for 30 min at 37°C and alkylated with 10 mM Iodacetamide at room temperature. The peptides were purified using C18 microspin columns (Harvard Apparatus) according to the protocol of the manufacturer, resolved in 0,1 % formic acid, 1 % acetonitrile and injected into the mass spectrometer. The samples were analysed on an LTQ-FT-ICR mass spectrometer (Thermo-Fischer Scientific), which was connected online to a nanoelectrospray ion source (Thermo-Fischer Scientific). Peptide separation was carried out using an Eksigent Tempo nano LC 1D+ System (Eksigent Technologies) equipped with a RP-HPLC column (75 µm x 15 cm) packed in-house with C18 resin (Magic C18 AQ 3 µm; Michrom BioResources) using a linear gradient from 96 % solvent A (0,15 % formic acid, 2 % acetonitrile) and 4 % solvent B (98 % acetonitrile, 0,15 % formic acid) to 25 % solvent B over 60 minutes at a flow rate of 0,3 µl/min. 4 µl of each sample were injected per LC-MS/MS run and analyzed. The data acquisition mode was set to obtain one high resolution MS scan in the ICR cell at a resolution of 100,000 full width at half maximum (at *m*/*z* 400) followed by MS/MS scans in the linear ion trap of the three most intense ions (overall cycle time of 1 second). To increase the efficiency of MS/MS attempts, the charged state screening modus was enabled to exclude unassigned and singly charges ions. Only MS precursors that exceeded a threshold of 150 ion counts were allowed to trigger MS/MS scans. The ion accumulation time was set to 500 ms (MS) and 250 ms (MS/MS) using a target setting of 10^6 (for MS) and 10^4 (for MS/MS) ions. After every sample, a peptide mixture containing 200 fmol of [Glu1]-Fibrinopeptide B human (Sigma) was analyzed by LC-MS/MS to constantly monitor the performance

of the LC-MS/MS system. Acquired MS2 scans were searched against the human International Protein Index (IPI) protein database (v.3.26) using the XTandem search algorithm (Craig & Beavis, 2004) with k-score plug-in (MacLean et al, 2006). In silico trypsin digestion was performed after lysine and arginine (unless followed by proline) in fully tryptic peptides. Allowed monoisotopic mass error for the precursor ions was 3 Daltons for the LTQ data and 50 ppm for the FT data. A fixed residue modification parameter was set for carboxyamidomethylation (+57.021464 Da) of cysteine residues. Oxidation of methionine (+15.994915 Da) was set as variable residue modification parameter. Model refinement parameters were set to allow phosphorylation (+79.966331 Da) of serine, threonine and tyrosine residues as variable modifications. Furthermore semi tryptic peptides were allowed for refinement searches. For scoring, a maximum of two missed cleavages were considered. Search results were evaluated on the Trans Proteomic Pipeline (TPP v3.2) using PeptideProphet (Keller et al, 2002) and ProteinProphet (Keller et al, 2005; Nesvizhskii et al, 2003).

For assays performed using Ska1, Ska2 or C13orf3 as baits the tryptic peptides were concentrated and desalted off-line using an UltraMicroSpin-C18 (The Nest Group) cartridge. Peptides were eluted by 150 µl of 80:20 acetonitrile/water with 0,1 TFA, the eluate was dried down and re-dissolved in 10 µl of 0,1 % TFA. The peptide mixtures was loaded to a 5mm×300m i.d. trapping micro-column packed with C18 PepMAP100 5 µm particles (Dionex) of Ultimate 3000 nanoLC system (Dionex) at a flow rate of 20 µl/min. Upon loading and washing, peptides were eluted onto a 15 cm×75 µm i.d. nano-column, packed with C18 PepMAP100 3 µm particles (Dionex). The nanoLC was interfaced on-line to a LTQ Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific) via a robotic nanoflow ion source TriVersa (Advion BioSciences Ltd.) equipped with a LC coupler and a nanoESI chip with the diameter

of nozzles of 4,2 µm. The TriVersa source was operated at the ionization voltage of 1,7 kV and was controlled by Chipsoft 6.4 software (Advion Biosciences). MS/MS was performed in data-dependent acquisition (DDA) mode under the control of Xcalibur 2.0 software (Thermo Fisher Scientific). The DDA cycle consisted of a survey scan acquired in one microscan within the range of *m/z* 300-1600 performed under the target mass resolution of 60000 FWHM (full width at half-maximum) at Orbitrap analyzer under the target value of 1E06 ions. Survey scan was followed by MS/MS fragmentation of the four most abundant multiply charged precursors. Spectra were acquired in one microscan under the normalized collision energy of 35 % and target value of 1E04 ions in the linear ion trap (ion selection threshold 400 counts; precursor ions isolation width 4 amu). Activation parameter *q* = 0,25 and activation time of 30 ms were applied. Previously fragmented precursors were dynamically excluded for 90 s. LC-MS/MS runs saved as .raw files were converted to .mgf (MASCOT generic format) files using extract_msn utility from Bioworks 3.1 software (Thermo Fisher Scientific) and searched against an International Protein Index - Human database (68360 sequence entries; updated February, 2008) by MASCOT v. 2.1 software (Matrix Science Ltd.). Tolerances for precursor and fragment masses were set at 10 ppm and 0,6 Da, respectively; up to 1 missed cleavage was allowed; instrument profile was set to ESI-Trap; oxidation of methionine, acetylation of the N-terminal peptide and phosphorylation of serine, threonine and tyrosine were set as variable modification. MASCOT identifications were considered confident if hits were produced by matching of at least three MS/MS spectra each with peptide ions score above 20.

Reference:

Craig R, Beavis RC (2004) TANDEM: matching proteins with tandem mass spectra. *Bioinformatics* **20**(9)**:** 1466-1467

Keller A, Eng J, Zhang N, Li XJ, Aebersold R (2005) A uniform proteomics MS/MS analysis platform utilizing open XML file formats. *Mol Syst Biol* **1:** 2005 0017

Keller A, Nesvizhskii AI, Kolker E, Aebersold R (2002) Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. *Anal Chem* **74**(20)**:** 5383-5392

MacLean B, Eng JK, Beavis RC, McIntosh M (2006) General framework for developing and evaluating database scoring algorithms using the TANDEM search engine. *Bioinformatics* **22**(22)**:** 2830-2832

Nesvizhskii AI, Keller A, Kolker E, Aebersold R (2003) A statistical model for identifying proteins by tandem mass spectrometry. *Anal Chem* **75**(17)**:** 4646-4658