Appendix

Material and Methods

Cell culture

The cervix carcinoma cell line HeLaS3 and a H2B-GFP derivative [1] were a gift of E. Nigg (University of Basel) and were cultured in DMEM (PAA laboratories, E15-009 or Sigma-Aldrich, D5671) supplemented with 10% fetal bovine serum (FBS, PAA laboratories, A15-151), 1% L-glutamine (PAA laboratories, M11-004), 100 U/ml penicillin and 100 µg/ml streptomycin (PAA laboratories, P11-010). Cells were incubated at 37 °C with 5% CO_2.

siRNA mediated protein depletion

Cells were transfected with 40 nM siRNA pre-mixed with Oligofectamine (Life Technologies, 12252-011) in Optimem (Life Technologies 31985-054) with the following sequences: GI2: CGUACGCGGAAUACUUCGAdTdT [2] Becn1-I: CCACUCUGUGAGGAAUGCACAGAUAdTdT [3] Becn1-II: CAGUUUGGCACAAUCAAUAdTdT [4], Becn1-ER: GCUCAGUAUCAGAGAGAAUdTdT [5], Nek1-1: GGUCUGUUUGAUGCAAACAACCCAAdTdT and Nek1-2: ACAUCAGCAUCUUUAUGCCAAGAUUdTdT, both from [6] for a total of either 48 or 72 h.

Cell lysis and immunoblot

Cells were harvested either by trypsinization when asynchronous or in mitosis by selective shake-off. Samples were lysed in 50 mM Tris pH 8.0, 150 mM NaCl, 0.5% NP-40, 50 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF, one tablet protease inhibitors (EDTA free, Roche) per 10 ml and 30 µg ml^{-1} DNaseI (Sigma-Aldrich). Protein concentration was measured by Bradford analysis (500-0006, Bio-rad). Proteins were electro-blotted on AmershamTM HybondTM—ECL nitrocellulose membranes (GE Healthcare). Proteins of interest were detected using the following antibodies: rabbit anti GAPDH (Cell

Signaling, 2118, 1:5000), rabbit anti Beclin 1 (Cell Signaling, 3495, 1:1000), mouse anti CDC27 (clone 35/CDC27, 1:300), rabbit anti NUP88 (Cell Signaling, 13613, 1:1000).

Flow cytometry

To define the DNA content of asynchronously growing cells, HeLaS3 were fixed with 70% ethanol and stained for 30 min with propidium iodide 40 μg/ml at 37°C in the presence of RNAse A, 100 μg/ml. Cell preparations were analysed with FACS Calibur (Becton-Dickinson) and FlowJo Software (TreeStar Inc., Ashland, OR, USA).

Time-lapse video microscopy

HeLaS3-H2B-GFP cells were transfected with siRNA for a total of 48 h. 12 h after transfection cells were arrested for 24 h by a single 2 mM thymidine (Sigma-Aldrich, T1895) block, followed by release into Leibovitz 15 (Life Technologies, 21083-027) medium supplemented with 10 % FBS, 100 u/ml penicillin, 100 μg/ml streptomycin and imaged every 5 min by a screening microscope (Cell IQ SLF or Cell IQ MLF, Chip Man Technologies) with a 10X objective. Micrographs displayed in Figure 1D have been generated on a Leica DMI6000 B system equipped with Adaptive Focus Control, an N PLAN L 20x/0.35 dry objective, Hamamatsu Flash4 camera and a X-Cite exacte light source. Images were acquired at multiple positions every 3 min.

Immunofluorescence microscopy

To enrich for mitotic figures, HeLaS3 cells grown on coverslips were arrested for 24 h by a single 2 mM thymidine (Sigma-Aldrich, T1895) block, followed by release into fresh medium for 10 h prior to fixation. Cells were simultaneously fixed and permeabilized for 10 min at room temperature (RT) in PTEMF buffer, as described previously [7]. CENP-F was stained using a rabbit antibody (Bethyl, IHC-00065, 1:400) whereas kinetochores were counterstained using a CREST human antiserum [8]. Primary antibodies were detected using donkey anti-rabbit and donkey anti-human conjugated to Alexa 488 and Alexa 647 (Invitrogen), respectively. The DNA was stained with 1 μg/ml Hoechst 33342. Images were acquired on a SP5 confocal microscope

(Leica), using a 1.2 NA/63× water objective and LAS AF acquisition software (Leica). Maximum intensity projections of Z-stacks were generated with Fiji and further processed with Adobe Photoshop.

Microarray data generation and analysis

HeLaS3 Cells were transfected with either Becn1-II or Becn1-ER siRNAs for a total of 48 h. 12 h following the siRNA transfection cells have been arrested for 24 h by a single 2 mM thymidine (Sigma-Aldrich, T1895) arrest, followed by release in the presence of 1 µM Nocodazole (Sigma-Aldrich, M1404). 12 h later mitotic cells were harvested by selective shake off. The gene expression microarray data set was generated at the Expression Profiling Unit of the Medical University of Innsbruck following standard manufacturer's protocols. In brief, total RNA was extracted using TRIzol (Life Technologies) and RNA quality and integrity was verified using an Agilent 2100 Bioanalyzer. Five hundred ng of RNA per sample were processed to generate biotinylated hybridization targets using the Affymetrix GeneChip 3′ IVT Express kit and the Affymetrix GeneChip hybridization, wash, and stain kit. Resulting targets, fragmented and labeled RNA, were hybridized to the in total 6 Affymetrix GeneChip Human Genome U133 Plus 2.0 microarrays in an Affymetrix fluidic station 450 and scanned using the Affymetrix scanner 3000. Data analysis was performed in R (version 3.0.2) using packages from the Bioconductor project. Microarray raw data was pre-processed using the GCRMA method [9]. Raw p-values for the significance of differential expression were calculated using the moderated t-test [10] and adjusted for multiple hypothesis testing using the method from Benjamini and Hochberg [11]. Probe sets with an adjusted p-value < 0.01 and an absolute M-value (log2 fold-change) > 1 were considered significantly differentially expressed (i.e. requiring a more than 2-fold differential expression at a 1% false discovery rate for a probe set to be considered differentially expressed).

Raw and pre-processed microarray data have been deposited to the Gene Expression Omnibus (GEO accession GSE69052). The link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=kxeloiiifviftqp&acc=GSE 69052.

Identification of potential siRNA off-targets

RNAplex [12] was used to calculate the binding energies between either siRNA and all transcripts' mRNAs for all protein coding and non-coding genes defined in the Ensembl database (version 75). For each gene the alignment with highest binding energy (considering all of its transcripts) was reported.

Supplementary References

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