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Supplemental Information

Characterizing ZC3H18, a Multi-domain Protein at the Interface of RNA Production and Destruction Decisions

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

AC experiments

For AC, 50-100mg of cell powder or cell pellet from ~10M cells were resuspended in 1ml respective extraction buffer (1. 20mM HEPES pH=7.4, 0,5% Triton X-100, 100mM NaCl; 2. 20mM HEPES pH=7.4, 0,5% Triton X-100, 300mM NaCl; 3. 20mM HEPES pH=7.4, 0,5% Triton X-100, 500mM NaCl; 4. 40mM Tris-Cl pH=8, 2mM EDTA, 1%SDS; choice of buffer specified in the text), containing Protease Inhibitor Cocktail (Roche) and, when indicated, Protein Phosphatase Inhibitors (Roche). Short sonication (2x5s) was applied in order to reduce viscosity. Cell lysate was clarified by 14000rpm centrifugation for 10min at 4°C. Next, protein extract (supernatant) was transferred to a tube containing 10µl of magnetic beads coupled to α-FLAG antibodies (SIGMA). Beads were pre-washed 3x with 1ml extraction buffer. Binding was performed on a rotator wheel for 1h in the cold. Subsequently, beads were washed 3x with 1ml extraction buffer. For RNase A/T1 treated samples beads were resuspended after washing in 20µl extraction buffer and 0,5µl RNAse A/T1 (Thermo Scientific). Samples were incubated for 15min at RT with shaking, washed 3x with 500µl extraction buffer and eluted. Elution was performed with 18µl of 1,1x NuPage Lithium dodecyl sulphate (LDS) Sample Buffer (Invitrogen) for 10min at RT with shaking. Reducing agent (DTT) was added to eluates to a final concentration of 50mM. Samples were heated at 75°C for 10min and loaded on NuPage 4-12% Bis-Tris gels (Invitrogen). Gels were stained with a Coomassie based Blue Silver stain (Candiano et al., 2004).

Interaction screening

Briefly, 50mg of cell powder was used for each AC condition and thawed in 450µl of appropriate extraction buffer containing Protease Inhibitor Cocktail (Roche) (composition of buffers can be found in Table S3). To ensure an even distribution of powder a 30sec sonication was applied using a QSonica Sonicator 4000 with an 8-microtip probe and amplitude setting of 15. Extracts were clarified by 14.000g centrifugation at 4°C for 10min and transferred to 96-well plates containing 5µl/well of pre-washed magnetic beads coupled to α-FLAG antibodies. Incubation was carried out for 1h while rotating in the cold and placed on a magnet to remove supernatants using a multi-channel pipette. Next, beads were washed with 2x500µl and 1x250µl of extraction buffer. When performing the final wash, beads were transferred to a fresh plate. From here elution was performed with 18ul of 1,1xLDS for 10min at RT with shaking. Eluates were transferred to a plate containing 2µl of reducing agent (final 50mM DTT). Samples were heated at 75°C and loaded on 26-well NuPage 4-12 % Bis-Tris gels (Invitrogen), which were stained with Blue-Silver stain.

MS analysis

Only protein hits detected in both replicates were included and hits with a ratio of single peptide intensity values higher than 10 or lower than 1/10 were discarded. Protein abundance was calculated as a ratio between a protein's mean peptide intensity from two experiments and its molecular weight and normalized to the abundance of the ZC3H18-3xF bait protein. RNase A/T1 resistance was calculated as the ratio between protein abundance in RNase A/T1–treated and untreated samples. Scatter plots were made using GraphPad Prism software.

Gel bands were digested overnight with 40µl 3,1ng/µl trypsin (Promega) in 25mM ammonium bicarbonate. An equal volume of 2,5mg/ml POROS R2 20µm beads (Life Technologies) in 5% v/v formic acid, 0,2% v/v TFA was added, and the mixture incubated on a shaker at 4°C for 24h. Digests were desalted on C18 resin (Empore), eluted, and dried by

vacuum centrifugation. Depending on band intensity, 1/20 – 1/3 of each sample was injected per LC-MS/MS analysis. Samples were loaded onto an Easy-Spray column (ES800, Thermo Fisher Scientific) and gradient-eluted (Solvent A = 0,1% v/v formic acid in water, Solvent B = 0,1% v/v formic acid in acetonitrile, flow rate 300nl/min) over 10 minutes into a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) acquiring data-dependent HCD fragmentation spectra.

Western blotting analysis

Protein extracts were run on NuPage 4-12% Bis-Tris gels (Invitrogen). Proteins were transferred from the gel to a PVDF membrane, 0.45µm (Millipore) by wet transfer with XCell II Blot Module (Invitrogen) for 2,5h at 4°C. After transfer, membranes were blocked for 1h with 5% non-fat dry milk dissolved in PBST buffer (10 mM Tris-HCl, pH 8.0; 0.15 M NaCl; 0.05% Tween 20). Next, membranes were incubated overnight at 4°C or for 1h at RT with dedicated primary antibodies (Table S4) diluted in 5% milk followed by incubation with the relevant secondary antibodies (Table S4) conjugated to horse radish peroxidase (HRP) diluted in PBST. Detection was performed using Supersignal West Femto Substrate (Thermo Scientific) according to the manufacturer's instructions

Cloning

To produce ZC3H18 fragments, truncated sequences were amplified by PCR, using primers listed in Table S6 and the following PCR program parameters: 1) initial denaturation at 98°C for 30sec, 2) 35 cycles of 98°C for 10sec, 72°C for 3min, 3) final extension at 72°C for 10min, 4) hold at 4°C. PCR products were run on 1% agarose gel and bands of correct size were cut out and purified using GenJet gel extraction kit (Thermo Scientific). Fragments were cloned into pcDNA5/FRT/TO_3xF(C) vector using HindIII and EcoRV restriction sites and subsequent ligation with T4 DNA ligase. Correct DNA sequence of final constructs was confirmed by Sanger sequencing.

Immunolocalization analysis

HeLa cells were grown on cover slips in 6-well plates in DMEM with 10% FBS. Cells were transfected with 2µg of plasmid constructs. 24h after transfection, cells were washed once with cold PBS and fixed in 4% para-formaldehyde for 20min at RT. After fixation, cells were permeabilized and blocked with PBS, 3% FBS, 0,5% Triton X-100 for 15min at RT. Cells were then washed 3 times for 5min with PBS, 3% FBS at RT and incubated with rabbit α-FLAG antibody (SIGMA, F7425) for 1h (1/1000 in PBS, 3% FBS) followed by incubation with goat α-rabbit Alexa Flour 488 (Life Technologies, A-11008) for 1h (1/1000). To visualize DNA, 1µg/µl 4′ 6-diamidino- 2-phenylindole (DAPI) was added for 10min at RT. After 3 washes in PBS at RT, cover slips were mounted on glass slides using Slow Fade Gold Antifade Mountant (Invitrogen). Cells were observed under a Zeiss 40x objective. Images were acquired using MetaMorph software and analyzed in ImageJ.

ChIP

HEK293 Flp-In T-REx cells were crosslinked with 1% formaldehyde for 10min at RT with mild agitation. Reactions were quenched by the addition of glycine to 0,125M. Cells were then washed in ice-cold PBS and lysed 10min in ChIP lysis buffer (20mM Tris-HCl, pH=8, 85mM KCl, 0,5% NP-40) on ice. Nuclei were pelleted and lysed >1h on ice in 1ml nuclei lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl, pH=8). Nuclear extracts (NE) were sonicated with a

Covaris sonicator for 15min at intensity 8, 20% burst and 200 cycles per burst. Debris was pelleted by centrifugation at 15.700g for 20min at 4°C, and aliquots were taken for estimation of DNA fragmentation efficiency by agarose gel electrophoresis. DNA concentration in sonicated NEs was measured and adjusted in nuclei lysis buffer, and equal amounts of DNA were diluted to 300µg/ml in ChIP dilution buffer (0,01% SDS, 1,1% Triton X-100, 1,2mM EDTA, 16,7mM Tris-HCl, pH=8, and 167mM NaCl). 1% aliquots were taken for 'input' samples, and 1mL NEs aliquots were incubated for 1,5h at 4°C with sheep α-rabbit beads (Invitrogen) coupled to 15µg of ZC3H18 antibody (SIGMA). As a negative control, the NE was incubated with beads only. After incubation, beads were washed once in low-salt buffer (0,1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH=8, and 150mM NaCl), once in high-salt buffer (0,1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH=8, and 500mM NaCl), once in LiCl immune-complex wash buffer (0,25M LiCl, 1% NP-40, 1% sodium deoxycholate, 1mM EDTA and 10mM Tris-HCl, pH=8) and twice in TE buffer (10mM Tris-HCl, pH=8, and 1mM EDTA). DNA was eluted twice in 100µl elution buffer (1% SDS, and 0,1M NaHCO3) by shaking for 10min at 65°C. Eluted DNA and input samples were reverse crosslinked by treatment with RNase A (overnight at 65°C) and Proteinase K (3h at 45°C), and DNA was purified on PCR-purification columns (Fermentas) and diluted to 200µl in nfH2O. qPCR reactions were set up in a total volume of 15µl with 5µl DNA sample, 2,5µM primer pair and 7,5µl 2× Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and run as a standard short PCR program with an annealing temperature of 60°C.

Annotations used for RNAseq and ChIPseq analysis

All annotations used were from the human genome reference assembly hg19. Gene annotations were taken from the Refseq release of hg19 (GRCh37) v3 (ftp://ftp.ncbi.nlm.nih.gov/genomes/Homo_sapiens/ARCHIVE/BUILD.37.3/GFF/ref_GRCh37.p5_top_level.gff3). Exon information was obtained from the same gff3 file selecting only exons assigned to a gene annotation (405045 exons from 28071 genes). Strictly intronic regions were obtained by collecting genes with exon annotations and subtracting all exonic parts from the same strand of each gene (229738 strictly intronic regions from 23479 genes).

RNAseq libraries

RNAseq data for siCBP80 depletion samples are first described here but were prepared in parallel with the described siEGFP, siARS2 and siZC3H18 samples (Iasillo et al., 2017) GEO: GSE99059. siRRP40, siZCCHC8 and siRBM7 and corresponding siEGFP control libraries are from (Meola et al., 2016) GEO: GSE84172.

RNAseq differential expression

Exonic counts were obtained using htseq-count from HTseq package with options (–s reverse –t exonic_part –m intersection-strict) using a gtf annotation file containing Refseq v3 exon information. Intronic counts were collected using htseq-count from the HTseq package v0.6.0 (Anders et al., 2015) with options (–s reverse –m intersection-strict –t intron), using a gtf annotation file containing strictly intronic. Exon and intron count tables were merged and used for differential expression analysis using the DESeq2 R package v1.10.1 (Love et al., 2014b). Since intronic regions are globally biased towards upregulation upon exosome depletion, only exonic reads were used for scaling (ie computation of sizeFactors). To avoid batch-specific artefacts, batch information was included in the DESeq2 design formula. Differential expression for depletion vs control was then computed using otherwise default settings.

Clustering analysis

Clustering of RNAseq data was based on log2FoldChange and padj measures from the DESeq2 analysis for exonic reads as described above. Genes with padj < .01; log2FoldChange > 1 or log2FoldChange < -1 for DESeq2 results for exonic reads in at least one or more of the depletion samples were selected (n=1587 genes). Correlation between samples was based on pearson correlation converted to distance using the formula (distance = $(-.5*r^2)-.5$) and plotted using R function heatmap.2 from package gplots v. 3.0.1 using agglomeration method 'ward.D2'.

ChIPseq analysis

ChIPseq data were obtained from GEO: GSE99344. For details on ChIPseq library preparation, sequencing, quality control, filtering and mapping see (Iasillo et al., 2017). ChIP libraries were background subtracted and scaled to gene body signals in protein-coding genes as described (Iasillo et al., 2017). log2FoldChanges between depletion and control samples were computed using these background-subtracted and scaled values for each replicate individually and the mean log2 ratio between the 2 replicates used for analysis in Figure 4. Only genes with valid signals in ChIPseq libraries and present in the RNAseq analysis were used for the final scatter plot (n=12,716 genes; thereof are n=1162 and n= 691 significantly (padj < .1) up- or down-regulated for intronic read analysis for RNAseq.

SUPPLEMENTAL REFERENCES

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics *31*, 166-169.

Candiano, G., Bruschi, M., Musante, L., Santucci, L., Ghiggeri, G.M., Carnemolla, B., Orecchia, P., Zardi, L., and Righetti, P.G. (2004). Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis *25*, 1327-1333.

Love, M.I., Huber, W., and Anders, S. (2014b). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology *15*, 550.

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

Figure S1

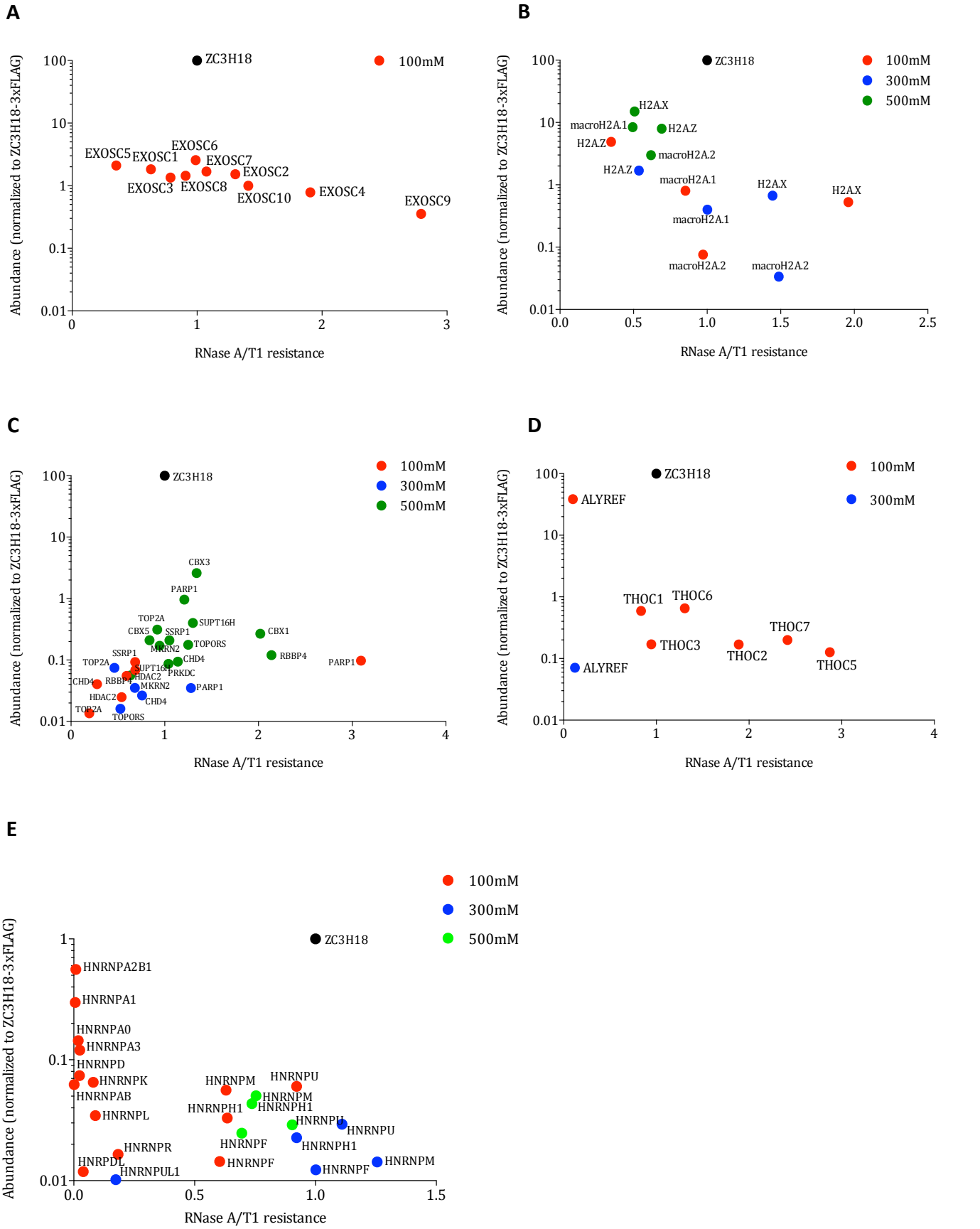

A
ZC3H18
100mM
EXOSC5
EXOSC1
EXOSC6
EXOSC7
EXOSC2
EXOSC3
EXOSC8
EXOSC10
EXOSC4
EXOSC9
Abundance (normalized to ZC3H18-3xFLAG)
RNase A/T1 resistance
B
ZC3H18
100mM
300mM
500mM
H2A.X
macroH2A.1
H2A.Z
macroH2A.2
Abundance (normalized to ZC3H18-3xFLAG)
RNase A/T1 resistance
C
ZC3H18
100mM
300mM
500mM
CBX3
PARP1
TOP2A
SUPT16H
CBX5
SSRP1
CBX1
TOPORS
MKRN2
RBBP4
CHD4
HDAC2
PRKDC
Abundance (normalized to ZC3H18-3xFLAG)
RNase A/T1 resistance
D
ZC3H18
100mM
300mM
ALYREF
THOC1
THOC6
THOC7
THOC3
THOC2
THOC5
Abundance (normalized to ZC3H18-3xFLAG)
RNase A/T1 resistance
E
ZC3H18
100mM
300mM
500mM
HNRNPA2B1
HNRNPA1
HNRNPA0
HNRNPA3
HNRNPD
HNRNPK
HNRNPAB
HNRNPL
HNRNPR
HNRPDL
HNRNPUL1
HNRNPM
HNRNPU
HNRNPH1
HNRNPF
Abundance (normalized to ZC3H18-3xFLAG)
RNase A/T1 resistance

Figure S1: MS analysis of co-purified proteins in ZC3H18-3xF AC experiments performed in 100, 300 and 500mM NaCl containing buffers. Related to Figure 1.

A) Scatter plot as in Figure 1B but presenting MS analysis of co-purified exosome components. Only the 100mM NaCl AC experiment is shown because exosome association was lost in higher salt conditions. The recovery of comparable amounts of all exosome subunits indicate co-purification of the full complex.

B) Scatter plot as in A) presenting MS analysis of co-purified non-canonical histone variants in the 100, 300 and 500mM NaCl containing buffers.

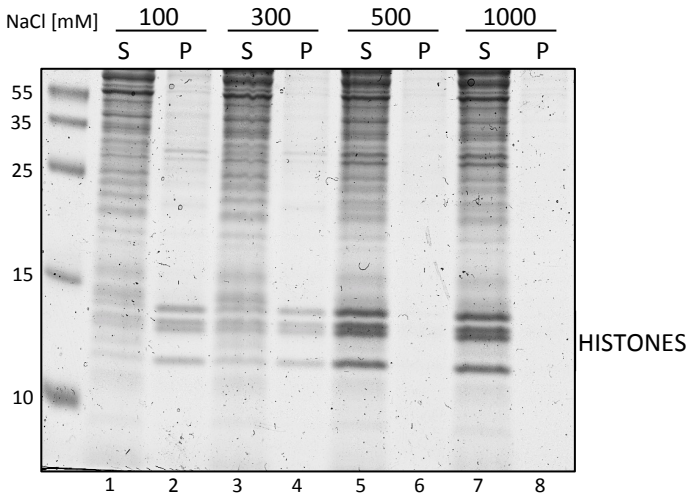
C) Scatter plot as in A) presenting MS analysis of co-purified DNA and chromatin related proteins in the 100, 300 and 500mM NaCl containing buffers.

D) Scatter plot as in A) presenting MS analysis of the co-purified TREX complex components in the 100 and 300mM NaCl containing buffers. Association with TREX was practically lost in the 300mM salt condition. Aly/REF, but not other TREX components, associates with ZC3H18 in an RNA-dependent manner.

E) Scatter plot as in A) presenting MS analysis of co-purified hnRNP proteins in the 100, 300 and 500mM NaCl containing buffers. Note that most hnRNPs form RNA-dependent interactions with ZC3H18-3xF.

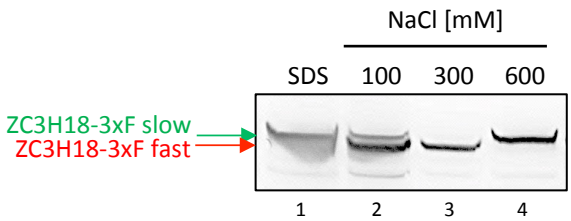
Figure S2

A



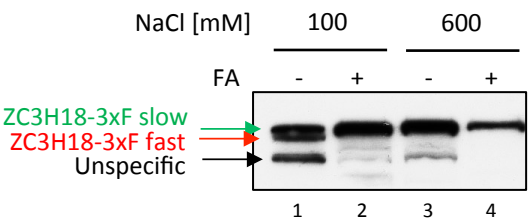
S – supernatant (soluble fraction)
P – pellet (insoluble fraction)

B



α-ZC3H18

C



α-ZC3H18

Figure S2: Histone solubility and presence of ZC3H18 forms in various extraction conditions. Related to Figure 2.

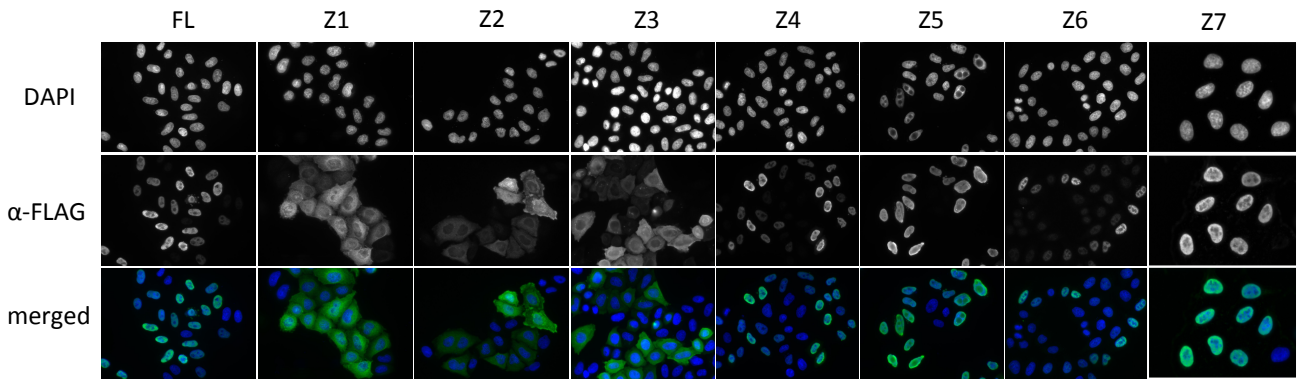
A) SDS-PAGE gel showing histone distribution between soluble (S) and pellet (P) fractions after protein extraction in buffers containing increasing NaCl concentrations as indicated. The migration of histones is indicated.

B) Western blotting analysis of protein extracts prepared in SDS- (40mM Tris-Cl pH=8, 2mM EDTA, 1%SDS) and NaCl- (20mM HEPES-Na pH=7,4, 0,5% Triton, 100, 300 or 600mM NaCl respectively) containing buffers. The membrane was probed with a α-ZC3H18 antibody. Note preservation of the high ZC3H18 isoform in both SDS- and 600mM NaCl-containing buffers (lanes 1 and 4). Slow and fast migrating ZC3H18 isoforms are denoted as in Figure 1.

C) Western blotting analysis of protein extracts prepared in 100 and 600mM NaCl-containing buffers from cells subjected to crosslinking with 1% FA (+) or not (-). The slow migrating ZC3H18 isoform is preserved upon fixation (compare lanes 1 and 2). An unspecific band is denoted by black arrow.

Figure S3

A



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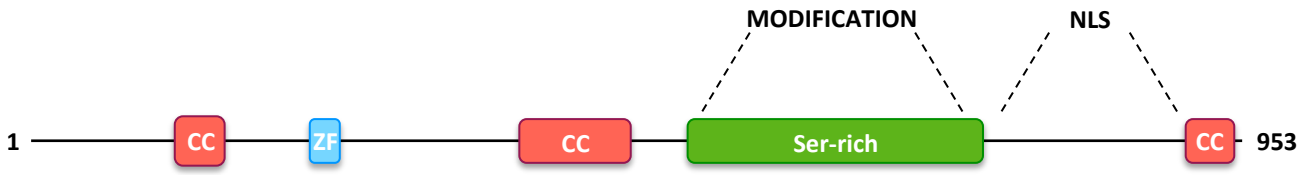

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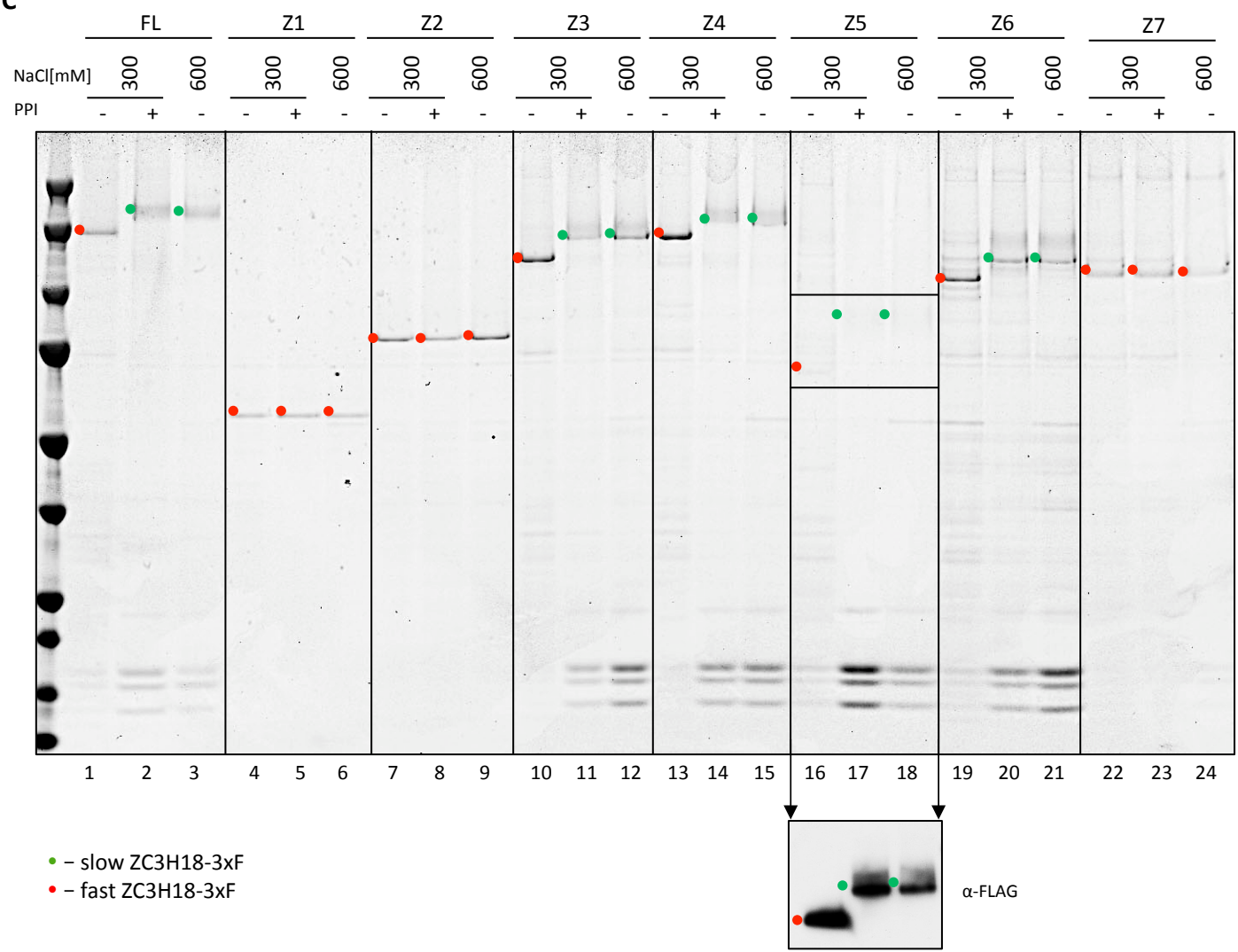


Figure S3: Localization and response to phosphatase inhibitors of ZC3H18-3F FL protein and mutants. Related to Figure 3.

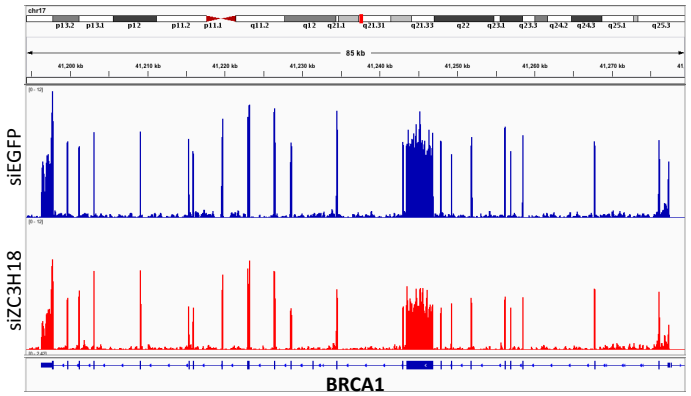
A) α -FLAG immunofluorescence microscopy analysis of HeLa Kyoto cells transiently expressing ZC3H18-3xF FL and mutants variants as indicated. 4 ,6-diamidino-2-phenylindole (DAPI) stain was included as a nuclear marker.

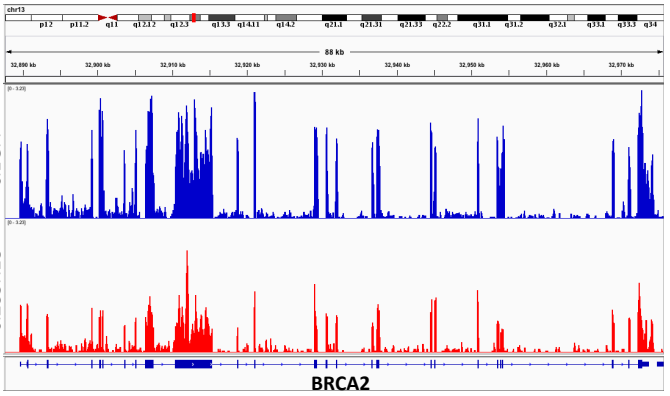
B) Schematic representation of ZC3H18 regions bearing nuclear localization signal (NLS) activity and post-translational modification capacity.

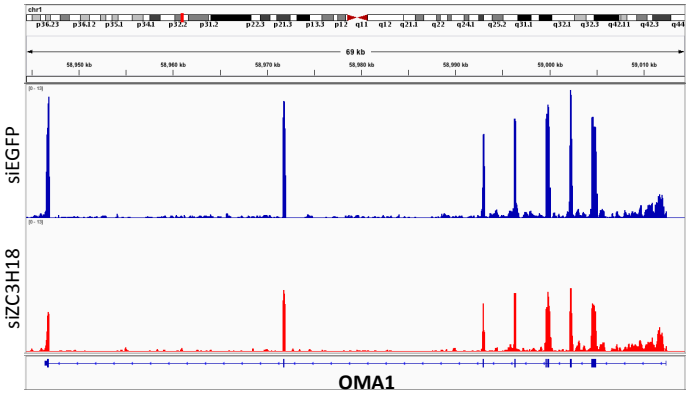
C) Coomassie stained SDS-PAGE gel of precipitated ZC3H18-3xF FL and mutant variants in 300 or 600mM NaCl containing buffers. The low salt extraction was additionally supplemented with PPI (+) or not (-). Fast and slow migrating isoforms of ZC3H18 are denoted with red and green dots, respectively. In case of the Z5 variant, precipitates were additionally visualized by α-FLAG western blotting analysis.

Figure S4

A







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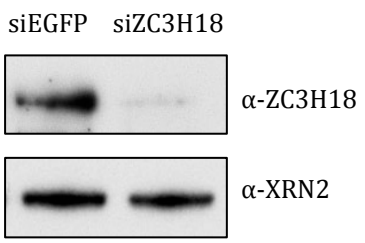

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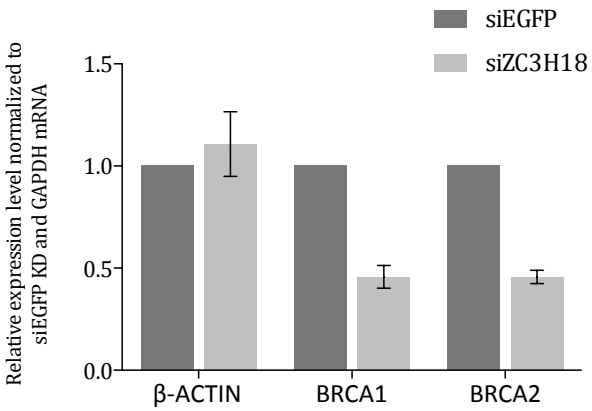

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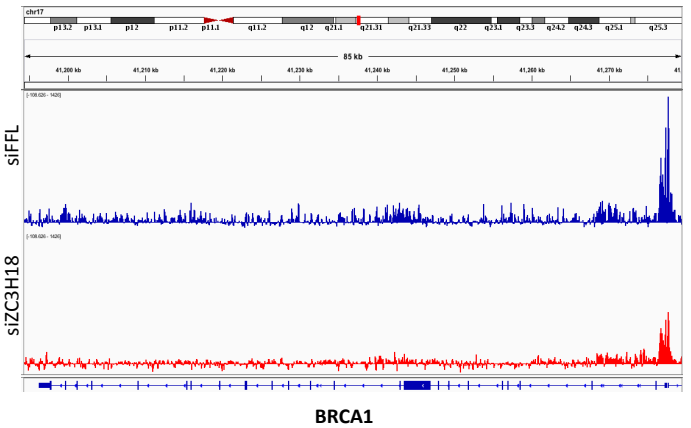
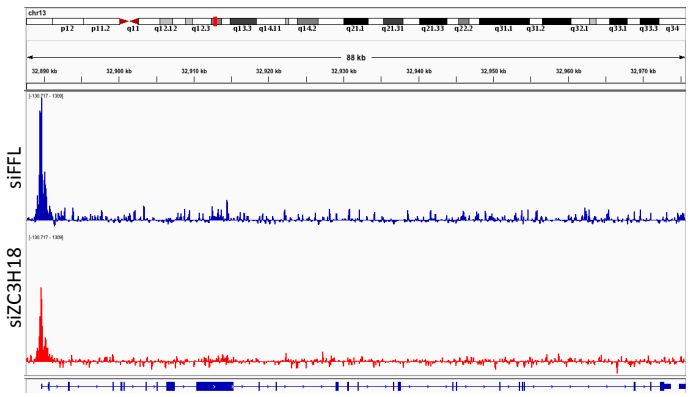




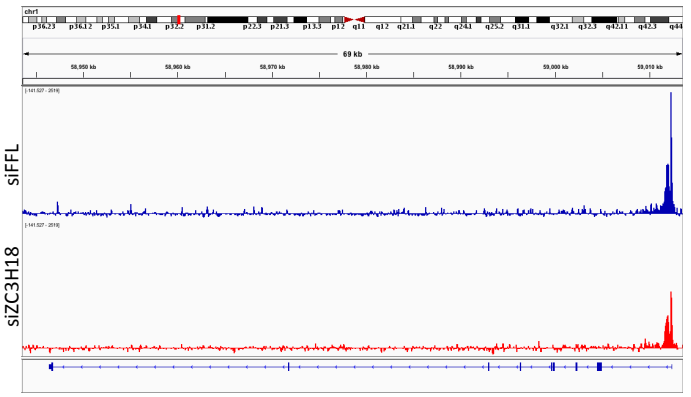


Figure S4: Genome browser screenshots and validation of ZC3H18-sensitive protein-coding genes. Related to Figure 4.

A) Genome browser screenshots showing *BRCA1*, *BRCA2* and *OMA1* downregulated gene expression upon ZC3H18 depletion. Triplicate RNA-seq data were pooled for EGFP control (top panels) and ZC3H18-depletion (bottom panels) samples. Only data for the same strand as the gene annotation are shown. Gene models below each panel show exon/intron structure.

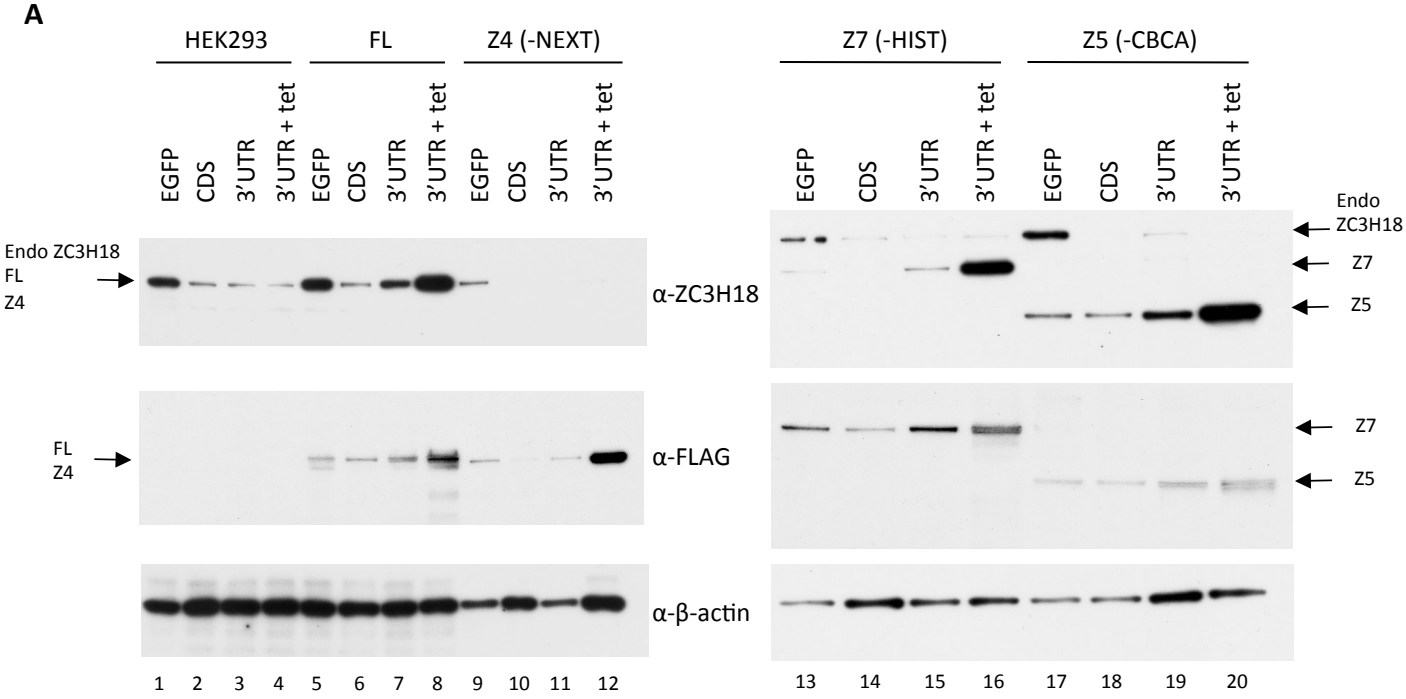
B) Western blotting analysis of HeLa Kyoto cell extracts depleted for ZC3H18. Control cells were treated with siRNAs against EGFP. α-XRN2 antibody was used as a loading control.

C) RT-qPCR analysis of total RNA from HeLa cells from B), using amplicons for *BRCA1* and *BRCA2* mRNAs. Data are displayed as avearge values of three biological replicates normalized to the control EGFP sample and normalized to GAPDH mRNA. Error bars represent standard deviations calculated from three biological replicates.

D) Genome browser screenshots showing decreased RNAPII occupancy over *BRCA1*, *BRCA2* and *OMA1* genes upon ZC3H18 depletion. Shown are pooled duplicate ChIP-seq data for FFL control (top panels) and ZC3H18-depletion (bottom panels) samples. Gene models below each panel show exon/intron structure. Due to the scaling, the effect appears dominant at TSS-proximal sites, however, gene bodies are affected as well.

Figure S5

A



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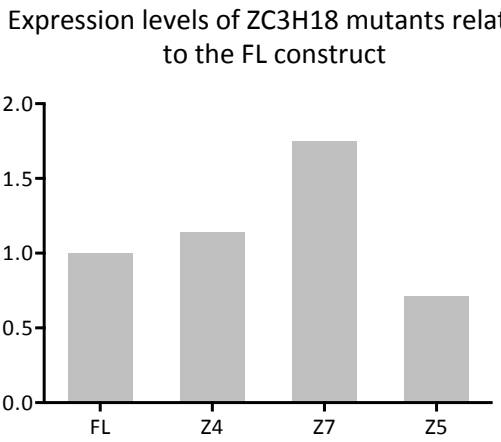

C

	BRCA2	BRCA1	OMA1	TP53	STAT5B	TMEM 45A	PTGR2	ZNF76	FAM 173B	LDAH	U11 3'ext	U12 3'ext
ZC3H18 KD vs. EGFP KD	0.0023	0.0003	0.0005	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0010	0.0045
ZC3H18 KD vs. FL rescue	0.0068	0.0092	0.0007	0.0002	0.0026	0.0001	0.0012	0.0001	0.0001	0.0001	0.0924	0.4568
ZC3H18 KD vs. Z5 rescue	0.8520	0.9997	0.9983	0.9392	0.9858	0.9997	0.6186	0.9997	0.9208	0.9996	0.2314	0.1185
ZC3H18 KD vs. Z4 rescue	0.1910	0.1038	0.0177	0.0075	0.4734	0.9816	0.7432	0.9980	0.6904	0.9997	0.9999	0.9819
ZC3H18 KD vs. Z7 rescue	0.0268	0.5500	0.9555	0.9998	0.0001	0.0001	0.0012	0.5801	0.9800	0.9998	0.3078	0.9999

Figure S5: Efficiency of endogenous ZC3H18 depletion and expression of ZC3H18-3xF FL protein and mutants. Related to Figure 5.

A) Western blotting analysis of ZC3H18 depletion efficiencies in the HEK293 parental cell line ('HEK293') and cell lines stably expressing ZC3H18-3xF FL and its mutant variants ('FL', 'Z4', 'Z7' and 'Z5'). Two different siRNAs were employed: i) 'CDS' – targeting the coding sequence and destabilizing mRNA produced from the endogenous ZC3H18 locus as well as from the FL, Z4 and Z7 exogenous loci, or ii) '3'UTR' – targeting the 3'UTR of only the endogenous ZC3H18 mRNA. Control cells were treated with EGFP siRNA ('EGFP'). After protein depletion, exogenous expression of fusion constructs was induced with 2ng/ml tetracycline ('tet' in lanes 4, 8, 12, 16 and 20). ZC3H18 protein levels were assessed using α-ZC3H18 and α-FLAG antibodies. The α-ZC3H18 antibody does not recognize the Z4 variant. Black arrows indicate bands corresponding to endogenous and exogenous ZC3H18 proteins. Due to their only minor size differences, ZC3H18-3xF FL, Z4 and endogenous ZC3H18 proteins could not be distinguished (left panel). Note that comparison between 'CDS'- and '3'UTR'-treated samples suggests some interference by 'leaky' fusion construct expression (compare lanes 6 and 7, 10 and 11, 14 and 15).

B) Quantification of the α-FLAG western blot from A). FLAG signal from samples 8, 12, 16 and 20 was first normalized to the respective α-actin signal and then to the FL value.

C) Table presenting p-values of differences between individual mRNA and snRNA levels in ZC3H18-depletion vs. 'rescued' samples calculated using a two-way ANOVA test. Color coding as in Figure 5B.

SUPPLEMENTAL TABLES

Table S1. MS analysis of ZC3H18-3xF AC experiments. Related to Figure 1.

Table S2. siRNA sequences. Related to Experimental Procedures.

Name	Sense strand sequence	Antisense strand sequence
siEGFP	GACGUAAACGGCCACAAGU	ACUUGUGGCCGUUUACGUC
siZC3H18_CDS	GGAAUGAAUUGUAGGUUUA	UAAACCUACAAUUCAUUCC
siZC3H18_3'UTR	CGACCGGACUGGACGCAUU	AAUGCGUCCAGUCCGGUCG

Table S3. Compositions of extractions buffers used in ZC3H18-3xF interaction screening. Related to Experimental Procedures.

Screening buffer #	Buffer composition
1	20mM HEPES-Na, pH 7.4, 100mM NaCl, 0,5% Triton
2	20mM HEPES-Na, pH 7.4, 200mM NaCl, 0,5% Triton
3	20mM HEPES-Na, pH 7.4, 300mM NaCl, 0,5% Triton
4	20mM HEPES-Na, pH 7.4, 500mM NaCl, 0,5% Triton
5	20mM HEPES-Na, pH 7.4, 1M NaCl, 0,5% Triton
6	20mM HEPES-Na, pH 7.4, 2M NaCl, 0,5% Triton
7	20mM HEPES-Na, pH 7.4, 100mM NaCl, 1% Triton
8	20mM HEPES-Na, pH 7.4, 200mM NaCl, 1% Triton
9	20mM HEPES-Na, pH 7.4, 300mM NaCl, 1% Triton
10	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1% Triton
11	20mM HEPES-Na, pH 7.4, 1M NaCl, 1% Triton
12	20mM HEPES-Na, pH 7.4, 2M NaCl, 1% Triton
13	20mM HEPES-Na, pH 7.4, 100mM NaCl, 5mM CHAPS
14	20mM HEPES-Na, pH 7.4, 200mM NaCl, 5mM CHAPS
15	20mM HEPES-Na, pH 7.4, 300mM NaCl,5mM CHAPS
16	20mM HEPES-Na, pH 7.4, 500mM NaCl, 5mM CHAPS
17	20mM HEPES-Na, pH 7.4, 1M NaCl, 5mM CHAPS
18	20mM HEPES-Na, pH 7.4, 2M NaCl, 5mM CHAPS
19	20mM HEPES-Na, pH 7.4, 500mM NaCl, 1M UREA, 1% Triton X-100
20	20mM HEPES-Na, pH 7.4, 500mM NaCl, 2M UREA, 1% Triton X-100
21	20mM HEPES-Na, pH 7.4, 500mM NaCl, 4M UREA, 1% Triton X-100

Table S4. Antibodies. Related to Experimental Procedures.

Name	Source	Cat# / reference	Working dilution
rabbit α-ZC3H18	SIGMA	HPA040847	1:4000
mouse α-β-ACTIN	SIGMA	A2228	1:100000

rabbit α-XRN2	Bethyl Laboratories	A301-102A	1:5000
rabbit α-ARS2	Abcam	ab88392	1:1000
rabbit α-CBP80	E. Izaurralde	(Izaurralde et al., 1994)	1:10000
rabbit α-MTR4	Abcam	ab70551	1:2500
rabbit α-RBM7	SIGMA	HPA013993	1:1000
rabbit α-ZCCHC8	Abcam	ab68739	1:1000
mouse α-FLAG	SIGMA	F1804	1:10000
goat α-rabbit IgG-HRP	DAKO	P0448	1:50000
goat α-mouse IgG-HRP	DAKO	P0447	1:50000

Table S5. Primers used in ChIP-qPCR analysis. Related to Experimental Procedures.

Name	Forward primer sequence	Reverse primer sequence
MYC +150	GGAGGGATCGCGCTGAGTA	TCTGCCTCTCGCTGGAATTAC
MYC +1400	GTTCCAGAACAGCTGCTAC	ACTCAATACGGAGATGCAA
MYC +5300	AAGTACATTTTGCTTTTTAAAGTTGATT	GGCTCAATGATATATTTGCCAGTTATTTTA
MYC -200	GTAGTTAATTCATGCGGCTCTCTTACT	GGGCAGCCGAGCACTCTA
MYC 1100	GTCCACAAGCTCTCCACTT	GTGCTAGACGGGAGAATATG
MYC -2000	GCTGGAAACTTGTTTTAAGG	TACTGGCAGCAGAGATCAT
GAPDH	CTCCTGTTCGACAGTCAGC	TTCAGGCCGTCCCTAGC
BRCA1	TGAGAGGCTGCTGCTTAGCG	TGAGAGGCTGCTGCTTAGCG
ZNHIT3	AGGCAAAAGCAATGGACTGA	AGGCAAAAGCAATGGACTGA
SNRPB2	AGGCAAAAGCAATGGACTGA	CTCCGTTTCCTGCTTTTGGT
PSMD3	CAGCTGCAAACCCCTTCC	CCTTTGTTGACTCGGCCATC
KRAS	CTCTTCCCTCTTCCCACACC	CTCTTCCCTCTTCCCACACC
MRPS15	GCTGCTAAACGTGGGCAC	GGATTAGGTGGCGTGACTCT
U12	GCAAAGTAGGCGGGTCAC	GACGCCCGAATCCTCACT
MYT1	CCCAAGGGTTCATGGGTAGC	CCTAAGCCAGCTAAAGGCGA
KCNA1	GAGAACGTGGACGAGGCTT	CTCGCAGCACTCGTGGTC
IFRG28	GCTCTTTATCTCTCTCTCAGCAAG	CAGTTTCGGTGTTCGGTTCA

Table S6. Primers used for PCR amplification of ZC3H18 fragments. Related to Experimental Procedures.

Name	Sequence
HindIII_Z18_N	ACTGAAGCTTACCATGGATGTGGCCGAG
Z18_Z1_C_EcoRV	CGTCCGGATATCCCATTCCAATCTCG
Z18_Z2_C_EcoRV	TGAGATATCCCGGCAGCCTCCTTCTTTG
Z18_Z3_C_EcoRV	TGAGATATCCCCGAATCCCTTTTCCGC

Z18_Z4_C_EcoRV	TGAGATATCCCCTTGCTGGAGCTCTTG
Z18_C_EcoRV	TGAGATATCCCTGCTTTCCCGGGGATC
HindIII_Z18_Z5_N	ACTAAGCTTACCATGGGGAAGCCCAAGCC
HindIII_Z18_Z6_N	ACGAAGCTTACCATGGACGATGATGACCTG
Z7_1	GAAGAAACTCGGGGTGCCGGCCCCAGCCCAG
Z7_2	CTGGGCTGGGGCCGGCACCCCGAGTTTCTTC

Table S7. Primers used for RT-qPCR. Related to Experimental Procedures.

Name	Forward primer sequence	Reverse primer sequence
GAPDH	GTCAGCCGCATCTTCTTTTG	GCGCCCAATACGACCAAATC
BRCA1	CAGCTGAGAGGCATCCAGAA	TCATGCTGTAATGAGCTGGCA
BRCA2	AAGCCCTTTGAGAGTGGAAG	TGGGCTCCATTTAGACCTGA
OMA1	AAAAGGGCTTCTGTCGTGAGT	CTTGATGTCGATTCCGCACG
TP53	CTGAACAAGTTGGCCTGCAC	CTCCCAAACATCCCTCACAGT
STAT5B	ACCGCTTGGGAGACTTGAAT	GTAGCAGACTCGCAGGGAAC
TMEM45A	CACACTCATGGCCGGGAAAT	GCCTGTCAGAAAGACGACCA
PTGR2	GACCTCAGAACTGGGCTTTGA	GGCATGATTCACGGAGCTGT
ZNF76	AGCAGTATGCCAGCAAGGTT	CCTTGTAGCCACAGCGGAAT
FAM173B	TCGCAGTACTCGAACGTTGT	AGGGAACCGGCAAGCAATAA
LDAH	GTCTGAGTCACCCAATGGCA	TGATTGTCTCAGGACACGGT
U11_3'END	ACGCGTTTGGAGTAAGTGGT	GGTCACCTGCGGTTCATACA
U12_3'END	TACTCAGGCTGCCAGTTGC	GCTGGGGACGGTGATGAAG