

# Supplemental Materials

*Molecular Biology of the Cell*

Ogawa et al.

**Supplemental Table S1.** The 113 high confidence screen hits that, when depleted, caused an increase in the percentage of nuclei with  $\geq 5$  nucleoli. Yeast ortholog,  $\geq 5$  nucleoli per nucleus normalized percent effect (NPE), percent viability, and nucleolar localization [1=NOPdb (Leung *et al.*, 2006; Ahmad *et al.*, 2009); 2=T-cell nucleolar proteome (Jarboui *et al.*, 2011); 3=Human Protein Atlas (Thul *et al.*, 2017)] indicated. The positive control, KIF11, was the top hit.

Screen hit (HGNC)	Yeast ortholog	$\geq 5$ nucleoli per nucleus NPE	Percent viability	Nucleolar (Y/N)
KIF11	Cin8/Kip1	68.75	19.00	N
CDCA8	Nbl1	66.59	12.91	Y [1, 3]
ASIC1		65.17	5.21	N
CMPK2	Cdc8	64.91	17.38	N
WBP11		64.64	12.61	Y [1]
KRT222		61.43	30.53	N
MICA		60.63	28.23	N
ATAD5	Elg1	59.75	27.67	N
RAP2C		57.83	38.96	N
SKP1	Skp1	57.64	13.00	Y [1]
TPX2		53.84	5.99	Y [2]
CIAO2B		47.53	26.97	N
PRRT2		47.35	21.61	N
RFFL		47.17	8.70	N
C11orf63		47.03	11.05	N
ZER1		46.73	43.72	N
MAN1A1	Mnl2	46.40	35.63	N
MFSD4		46.29	15.22	N
INCENP	Sli15	45.64	18.92	Y [1, 2]
SHC3		45.32	21.55	N
ENY2	Sus1	41.93	6.02	N
CUL1	Cdc53	41.61	36.50	Y [3]
SHROOM2		40.79	16.04	N
CTF1		40.36	8.66	N
MDN1	Rea1	39.82	29.07	Y [1, 2, 3]

ZDHHC17	Akr1/2	39.45	53.42	N
CDCA5		39.33	9.55	N
CASP8AP2		39.14	13.82	Y [2, 3]
HYPK		38.87	15.80	N
INKA1		38.64	14.41	N
NAT2		38.52	27.49	N
IRF2BP1		37.62	38.21	N
PMM2	Sec53	37.35	23.22	N
GLYATL2		36.68	28.51	N
TTC22		36.12	21.61	N
XRCC5	Yku80	35.61	42.14	Y [1, 2]
CYP4V2		35.61	58.17	N
RRM1	Rnr1/3	35.33	9.46	N
FAM221A		34.73	49.23	N
ECHDC2		34.49	22.43	N
RBBP8		33.82	21.42	N
CCDC81		33.78	22.74	N
KRBA1		32.89	29.14	N
PRUNE		32.76	19.24	N
SCRN3		32.28	63.05	N
AGR2		31.52	25.80	N
SMG5	Ebs1/Est1	31.03	27.66	N
LAPTM5		30.61	32.18	N
DBNDD1		30.51	10.27	N
RFC1	Rfc1	30.18	46.61	Y [1, 2]
IFT88		30.16	57.16	N
C9orf142		29.86	29.73	N
SGOL1		29.67	16.63	N
EBF3		29.63	36.33	N
MCM6	Mcm6	29.60	54.59	Y [1, 2]
OSBP2	Hes1/Kes1	29.58	14.65	N
PRAM1		29.56	14.07	N
GCNT2		29.56	34.99	N
ZNF219		29.31	45.34	N
LDB1		29.25	50.16	N

FAM58A		29.22	22.15	N
LIG3		29.07	40.91	Y [2]
SNX21		29.02	25.45	N
GLTP		28.95	20.74	N
GZF1		28.93	47.20	Y [3]
MAP4K5		28.92	27.53	N
SLC26A7		28.77	61.21	N
TARS2	Mst1	28.66	36.20	N
ABCE1	Rli1	28.57	25.65	Y [2]
LCORL		28.28	21.07	N
TYMP		28.06	72.51	N
MRPL52		27.86	36.04	N
RIMS3	Tcb3	27.82	10.82	Y [3]
WBSCR27		27.79	33.22	N
H1-10	Hho1	27.78	31.61	Y [1, 2, 3]
ZNF678		27.75	19.58	Y [3]
RACGAP1	Bem2/Rga1/2/Rgd1	27.62	15.60	N
STK24	Kic1/Pbs2	27.53	13.92	Y [3]
SLC2A12	Stl1	27.49	71.34	N
NGRN	Rrg9	27.47	25.68	N
LUC7L	Luc7	27.15	25.86	N
YIPF7	Yip1	27.11	5.29	N
WRAP53	Swt21	27.10	9.48	N
DYNC1H1	Dyn1	26.87	19.67	Y [1, 2]
GEN1		26.82	51.87	N
NR0B2		26.79	37.50	N
ANKEF1		26.73	22.43	Y [3]
KTN1		26.70	14.57	N
FGD4	Cdc24/Rom1/2	26.65	28.85	N
MIA		26.64	39.43	N
CCN4		26.52	46.48	N
GOLGA8EP		26.48	20.11	N
TOPBP1	Dpb11	26.37	10.58	Y [1]
SMAP2	Glo3/Gts1	26.24	33.90	N
OXNAD1	Aim33/Pga3	26.16	60.29	N

HSD11B2		26.09	21.32	N
MARCH9	Ssm4	26.06	68.46	N
TM2D1		26.04	19.78	N
PCOLCE2		26.02	19.56	N
CAMK2N1		25.99	26.33	N
RAPH1		25.95	6.16	N
MPV17L2	Mpv17	25.65	13.92	N
FAM98A		25.60	10.13	N
SUV39H1		25.56	32.30	N
TAF1D		25.54	27.66	N
NFYB	Hap3	25.50	41.60	N
MASTL	Pkh3/Rim15	25.41	32.46	N
GTF2IRD1		25.38	24.82	N
SCN2B		25.29	15.56	N
MLL3		25.28	73.70	N
DDAH1		25.27	55.36	Y [3]
NLRC5	Gip3/Her1	25.12	46.44	N
SMAD5		25.00	61.84	N

**Supplemental Table S2.** Molecular and cellular function core analysis in Ingenuity Pathway

Analysis (Qiagen) revealed a significant association with 23 categories

Category	$-\log_{10}$ p-value	# of hits	Hits
Cell cycle	5.66	31	CAMK2N1, CASP8AP2, CDCA5, CDCA8, CIAO2B, CUL1, DYNC1H1, ENY2, GEN1, HYPK, INCENP, KIF11, LIG3, MASTL, MCM6, MIA, NR0B2, RACGAP1, RBBP8, RFC1, RFFL, RRM1, SGO1, SHROOM2, SKP1, SUV39H1, TAF1D, TOPBP1, TPX2, XRCC5, ZNF219
Cellular Assembly and Organization	4.95	24	CDCA5, CDCA8, CIAO2B, CUL1, DYNC1H1, FGD4, GEN1, IFT88, INCENP, JHY, KIF11, LIG3, MIA, OSBP2, PRUNE1, RACGAP1, RAPH1, RBBP8, RIMS3, SGO1, SUV39H1, TOPBP1, TPX2, XRCC5
DNA Replication, Recombination, and Repair	4.95	27	ATAD5, CDCA5, CIAO2B, CMPK2, CUL1, DYNC1H1, ENY2, GEN1, INCENP, KIF11, LIG3, MCM6, MICA, PAXX, RACGAP1, RBBP8, RFC1, RRM1, SGO1, STK24, SUV39H1, TOPBP1, TPX2, TYMP, WRAP53, XRCC5, ZNF219

Cell Morphology	3.32	16	CTF1, FGD4, IFT88, KIF11, LDB1, OSBP2, RACGAP1, RAPH1, RBBP8, RIMS3, SMAD5, SUV39H1, TOPBP1, TPX2, TYMP, XRCC5
Nucleic Acid	2.91	4	CMPK2, PMM2, RRM1, TYMP
Small Molecule Biochemistry	2.91	13	CMPK2, DYNC1H1, GLYATL2, HSD11B2, KMT2C, NAT2, NR0B2, OSBP2, PMM2, RAPH1, RRM1, SUV39H1, TYMP
Molecular Transport	2.69	6	KMT2C, NR0B2, RACGAP1, RAPH1,
Cellular Function and Maintenance	2.42	17	CDCA5, DYNC1H1, FGD4, IFT88, JHY, KIF11, MIA, PRUNE1, RAPH1, RBBP8,
Cell Death and Survival	2.42	36	ABCE1, AGR2, ASIC1, ATAD5, CASP8AP2, CCN4, CDCA5, CTF1, CUL1, DYNC1H1, FGD4, HSD11B2, HYPK, IFT88, INCENP, KIF11, LIG3, MDN1, MIA, MICA, NR0B2, OSBP2, PAXX, RACGAP1, RFC1, RRM1, SHC3, SHROOM2, SMAD5, STK24, SUV39H1, TM2D1, TOPBP1, TPX2, TYMP, XRCC5
Cell-To-Cell Signaling	2.32	5	ASIC1, GTF2IRD1, HSD11B2, IFT88, MICA
Cellular Compromise	2.32	9	ASIC1, CTF1, CUL1, DYNC1H1, KIF11,
Cellular Development	2.32	15	CCN4, CDCA5, CTF1, CUL1, EBF3, IFT88, MIA, OSBP2, PRUNE1, RAPH1, RFC1, RIMS3, SMAD5, STK24, SUV39H1

Cellular Growth and Proliferation	2.32	16	CCN4, CDCA5, CTF1, CUL1, EBF3, HSD11B2, IFT88, LDB1, MIA, OSBP2, PRUNE1, RAPH1, RFC1, RIMS3, SMAD5, SUV39H1
Cellular Movement	2.32	5	CCN4, FGD4, IFT88, INCENP, MIA
Cellular Response to	2.32	2	PAXX, XRCC5
Drug Metabolism	2.32	3	CMPK2, HSD11B2, NAT2
Lipid Metabolism	2.32	7	DYNC1H1, GLYATL2, HSD11B2, KMT2C,
Amino Acid	2.02	2	GLYATL2, SUV39H1
Carbohydrate	2.02	2	PMM2, RAPH1
Gene Expression	2.02	3	LIG3, NR0B2, SUV39H1
Post-Translational	2.02	4	FGD4, GLYATL2, MAP4K5, SUV39H1
Vitamin and Mineral	1.84	1	NR0B2
Cell Signaling	1.70	2	FGD4, MAP4K5



**Supplemental Table S3.** Nuclear area analysis of screen hit depletions comparing nuclei with 0-4 nucleoli to nuclei with  $\geq 5$  nucleoli. For each screen hit depletion, nuclei were classified as either nuclei with 0-4 nucleoli or  $\geq 5$  nucleoli and the average nuclear area was calculated (n=3 for hits; n=6 for controls). Significance was determined by unpaired t-tests and based on a False Discovery Rate approach using the two-stage step-up method of Benjamini, Krieger and Yekutieli (n=3 or 6;  $q < 0.01 / -\log q\text{-value} > 2$ ). siRISC-free=negative control. siKIF11 (pool)=screen positive control.

Screen Hit (siRNA HGNC, - last 2 digits of product number)	Nuclear area 0-4 nucleoli (mean $\pm$ SD)	Nuclear area $\geq 5$ nucleoli (mean $\pm$ SD)	Significance (q-value)
siRISC-free	206.4 $\pm$ 2.9	365.7 $\pm$ 6.5	q<0.0001
siKIF11 (pool)	435.8 $\pm$ 10.1	666.9 $\pm$ 18.1	q<0.0001
siKIF11 -05	382.2 $\pm$ 10.4	572.5 $\pm$ 14.5	q<0.0001
siKIF11 -06	374.9 $\pm$ 11.9	640.1 $\pm$ 29.5	q<0.001
siKIF11 -07	409.2 $\pm$ 16.8	676.5 $\pm$ 26.3	q<0.001
siKIF11 -08	430.4 $\pm$ 22.1	678.7 $\pm$ 28.5	q<0.001
siABCE1 -01	345.6 $\pm$ 15.5	567.6 $\pm$ 22.4	q<0.001
siABCE1 -02	244.4 $\pm$ 3.5	403.3 $\pm$ 6.5	q<0.0001

siABCE1 -04	270.8 ± 4.6	404.7 ± 11.5	q<0.0001
siABCE1 -17	243.8 ± 6.0	374.3 ± 3.7	q<0.0001
siATAD5 -01	342.5 ± 11.0	503.6 ± 23.5	q<0.001
siATAD5 -02	245.6 ± 3.2	356.4 ± 8.2	q<0.0001
siATAD5 -03	264.5 ± 7.1	411.7 ± 8.2	q<0.0001
siATAD5 -04	262.2 ± 5.9	417.5 ± 21.0	q<0.001
siCCN4 -01	254.2 ± 6.1	437.5 ± 5.7	q<0.0001
siCCN4 -02	359.6 ± 30.2	551.9 ± 57.1	q<0.01
siCCN4 -03	294.4 ± 6.8	417.0 ± 7.5	q<0.0001
siCCN4 -17	347.8 ± 7.6	519.7 ± 11.3	q<0.0001
siCDC48 -01	318.2 ± 21.5	892.2 ± 1.5	q<0.0001
siCDC48 -02	342.4 ± 5.0	887.7 ± 36.1	q<0.0001
siCDC48 -03	301.8 ± 3.9	634.8 ± 12.7	q<0.0001
siCDC48 -04	322.3 ± 8.6	728.9 ± 4.6	q<0.0001
siCIAO2B -01	286.0 ± 5.4	412.7 ± 3.2	q<0.0001
siCIAO2B -03	277.8 ± 9.4	446.7 ± 21.1	q<0.001
siCIAO2B -04	270.5 ± 1.9	445.7 ± 4.1	q<0.0001

siCIAO2B -18	305.7 ± 4.4	433.4 ± 16.5	q<0.001
siDYNC1H1 -01	311.5 ± 2.3	464.9 ± 13.1	q<0.0001
siDYNC1H1 -02	296.8 ± 2.4	511.1 ± 6.1	q<0.0001
siDYNC1H1 -03	427.8 ± 2.6	644.3 ± 4.9	q<0.0001
siDYNC1H1 -04	271.8 ± 5.5	483.0 ± 3.6	q<0.0001
siENY2 -01	284.6 ± 3.5	507.5 ± 12.6	q<0.0001
siENY2 -02	254.6 ± 5.3	446.6 ± 15.3	q<0.0001
siENY2 -03	272.8 ± 1.8	464.5 ± 11.5	q<0.0001
siENY2 -04	307.5 ± 6.2	479.5 ± 1.7	q<0.0001
siFAM98A -01	395.4 ± 5.7	663.7 ± 15.4	q<0.0001
siFAM98A -02	357.8 ± 24.9	554.2 ± 27.6	q<0.001
siFAM98A -03	206.6 ± 3.9	386.6 ± 9.8	q<0.0001
siFAM98A -04	329.4 ± 4.2	505.3 ± 14.8	q<0.0001
siH1-10 -01	274.9 ± 10.2	444.9 ± 16.8	q<0.001
siH1-10 -02	313.8 ± 4.2	493.6 ± 9.2	q<0.0001
siH1-10 -03	244.2 ± 4.9	392.5 ± 8.5	q<0.0001
siH1-10 -04	323.7 ± 6.4	514.4 ± 37.9	q<0.01

siINCENP -01	340.5 ± 9.4	832.2 ± 13.0	q<0.0001
siINCENP -02	426.7 ± 14.7	788.9 ± 10.0	q<0.0001
siINCENP -03	449.7 ± 8.8	886.4 ± 6.5	q<0.0001
siINCENP -04	355.5 ± 6.2	672.4 ± 13.8	q<0.0001
siINKA1 -01	417.5 ± 7.3	630.3 ± 9.4	q<0.0001
siINKA1 -02	262.5 ± 3.4	488.3 ± 13.9	q<0.0001
siINKA1 -03	258.9 ± 8.2	382.9 ± 1.4	q<0.0001
siINKA1 -04	377.2 ± 5.9	579.2 ± 12.5	q<0.0001
siKTN1 -17	301.1 ± 16.8	467.2 ± 7.8	q<0.001
siKTN1 -18	364.0 ± 3.1	532.1 ± 11.4	q<0.0001
siKTN1 -19	216.3 ± 3.0	375.5 ± 5.0	q<0.0001
siKTN1 -20	262.9 ± 3.8	420.5 ± 11.7	q<0.0001
siLUC7L -01	299.1 ± 8.4	451.4 ± 14.6	q<0.001
siLUC7L -02	278.5 ± 12.5	474.4 ± 27.6	q<0.001
siLUC7L -03	245.2 ± 3.2	379.3 ± 7.9	q<0.0001
siLUC7L -04	281.2 ± 12.3	424.0 ± 21.0	q<0.001
siMDN1 -03	251.2 ± 3.0	388.2 ± 12.3	q<0.0001

siMDN1 -17	343.7 ± 4.7	542.5 ± 14.4	q<0.0001
siMDN1 -18	280.5 ± 6.2	460.7 ± 8.9	q<0.0001
siMDN1 -19	279.3 ± 2.5	431.6 ± 10.9	q<0.0001
siRACGAP1 -01	318.7 ± 3.5	585.2 ± 6.8	q<0.0001
siRACGAP1 -02	285.1 ± 9.7	540.0 ± 5.5	q<0.0001
siRACGAP1 -03	328.5 ± 10.5	624.4 ± 13.5	q<0.0001
siRACGAP1 -04	300.5 ± 9.1	612.2 ± 28.2	q<0.0001
siRFC1 -01	268.2 ± 5.5	431.4 ± 6.8	q<0.0001
siRFC1 -02	288.4 ± 5.9	450.9 ± 3.2	q<0.0001
siRFC1 -03	288.9 ± 11.5	418.6 ± 12.7	q<0.001
siRFC1 -04	203.3 ± 1.5	412.1 ± 10.0	q<0.0001
siSTK24 -05	302.4 ± 4.7	479.9 ± 14.3	q<0.0001
siSTK24 -21	214.0 ± 0.8	385.5 ± 10.9	q<0.0001
siSTK24 -22	269.5 ± 3.2	427.1 ± 11.2	q<0.0001
siSTK24 -23	311.2 ± 3.1	439.1 ± 9.7	q<0.0001
siTPX2 -01	413.9 ± 25.9	641.0 ± 31.6	q<0.001
siTPX2 -02	367.8 ± 11.9	613.8 ± 24.1	q<0.001

siTPX2 -03	352.0 ± 4.8	559.8 ± 0.6	q<0.0001
siTPX2 -04	299.9 ± 2.2	570.1 ± 5.9	q<0.0001
siWRAP53 -19	211.7 ± 1.4	389.8 ± 4.1	q<0.0001
siWRAP53 -20	276.9 ± 2.9	407.9 ± 7.7	q<0.0001
siWRAP53 -21	279.4 ± 5.5	457.2 ± 17.4	q<0.0001
siWRAP53 -22	288.0 ± 1.8	440.0 ± 5.4	q<0.0001
siXRCC5 -01	243.6 ± 5.8	378.0 ± 3.6	q<0.0001
siXRCC5 -02	238.6 ± 1.1	389.1 ± 10.2	q<0.0001
siXRCC5 -03	218.0 ± 2.9	370.8 ± 6.5	q<0.0001
siXRCC5 -04	204.7 ± 2.2	370.7 ± 7.4	q<0.0001

**Supplemental Table S4.** Cell cycle analysis of screen hits selected for validation by oligonucleotide deconvolution. Each siRNA in the pool of 4 was tested independently (n=3). Controls, siRISC-free and the siKIF11 pool, were also assayed (n=48). The column with the number (No.) of cells is the total sum of nuclei analyzed in all replicates and is number of cells used to generate the cumulative histograms in Figure 5, **A**. Significance is listed in parentheses below the mean  $\pm$  SD and was determined by unpaired t-tests relative to siRISC-free and a False Discovery Rate approach using the two-stage step-up method of Benjamini, Krieger and Yekutieli (n=3 or 48; q<0.01=\*; q<0.001=\*\*; q<0.0001=\*\*\*; ns=not significant). A column that includes the  $\geq 5$  nucleoli per nucleus normalized percent effect (NPE) is also included. NPE in black=validated; NPE in red=not validated.

Screen Hit (siRNA HGNC, - last 2 digits of product number)	No. of cells (sum)	% G0/G1 (mean $\pm$ SD)	% S (mean $\pm$ SD)	% G2/M (mean $\pm$ SD)	% >4N (mean $\pm$ SD)	$\geq 5$ nucleoli per nucleus NPE
siRISC-free	498,155	48.3 $\pm$ 2.0	10.2 $\pm$ 1.1	20.3 $\pm$ 0.8	2.7 $\pm$ 0.5	0
siKIF11 (pool)	93,027	12.2 $\pm$ 1.2 (q<0.0001)	7.5 $\pm$ 0.8 (q<0.0001)	60.6 $\pm$ 1.7 (q<0.0001)	12.1 $\pm$ 1.1 (q<0.0001)	100
siKIF11 -05	3426	16.5 $\pm$ 1.9 (q<0.0001)	9.1 $\pm$ 1.2 (ns)	56.1 $\pm$ 2.5 (q<0.0001)	7.2 $\pm$ 1.8 (q<0.0001)	89.98
siKIF11 -06	6816	15.8 $\pm$ 1.0	8.7 $\pm$ 0.3	56.7 $\pm$ 0.8	9.9 $\pm$ 0.3	118.62

		(q<0.0001)	(q<0.01)	(q<0.0001)	(q<0.0001)	
siKIF11 -07	2154	23.8 ± 4.5 (q<0.0001)	10.8 ± 2.1 (ns)	42.1 ± 1.6 (q<0.0001)	6.9 ± 2.4 (q<0.0001)	74.75
siKIF11 -08	2721	20.6 ± 2.3 (q<0.0001)	10.5 ± 1.0 (ns)	50.7 ± 3.5 (q<0.0001)	7.3 ± 0.4 (q<0.0001)	116.70
siABCE1 -01	12653	30.6 ± 0.7 (q<0.0001)	20.0 ± 0.3 (q<0.0001)	33.6 ± 1.0 (q<0.0001)	8.3 ± 0.6 (q<0.0001)	50.71
siABCE1 -02	22023	42.7 ± 1.0 (q<0.0001)	15.4 ± 0.7 (q<0.0001)	25.9 ± 0.6 (q<0.0001)	5.8 ± 0.3 (q<0.0001)	22.63
siABCE1 -04	19958	49.9 ± 1.5 (ns)	23.3 ± 0.4 (q<0.0001)	15.9 ± 0.9 (q<0.0001)	1.8 ± 0.2 (q<0.01)	26.31
siABCE1 -17	21264	50.2 ± 2.9 (ns)	12.9 ± 0.8 (q<0.0001)	21.7 ± 1.6 (q<0.001)	1.9 ± 0.2 (q<0.01)	9.86
siATAD5 -01	11302	32.3 ± 14.3 (q<0.0001)	32.3 ± 11.6 (q<0.0001)	24.1 ± 1.6 (q<0.0001)	3.9 ± 2.4 (q<0.01)	55.60
siATAD5 -02	17992	49.9 ± 3.5 (ns)	19.4 ± 2.0 (q<0.0001)	22.3 ± 1.2 (q<0.0001)	1.5 ± 0.4 (q<0.001)	16.49
siATAD5 -03	21010	45.1 ± 1.9	17.2 ± 0.9	25.7 ± 1.1	2.7 ± 0.2	25.01



		(q<0.01)	(q<0.0001)	(q<0.0001)	(ns)	
siATAD5 -04	14337	37.0 ± 5.6 (q<0.0001)	19.8 ± 3.9 (q<0.0001)	27.8 ± 2.2 (q<0.0001)	4.1 ± 0.5 (q<0.0001)	22.60
siCCN4 -01	24641	45.9 ± 0.9 (q<0.01)	20.3 ± 1.8 (q<0.0001)	15.4 ± 0.4 (q<0.0001)	2.6 ± 0.2 (ns)	17.85
siCCN4 -02	4868	40.6 ± 4.3 (q<0.0001)	14.6 ± 2.0 (q<0.0001)	30.8 ± 3.3 (q<0.0001)	2.5 ± 0.6 (ns)	18.51
siCCN4 -03	7630	44.5 ± 1.7 (q<0.01)	13.7 ± 1.0 (q<0.0001)	27.8 ± 0.3 (q<0.0001)	2.0 ± 0.4 (ns)	23.89
siCCN4 -17	12550	35.7 ± 2.2 (q<0.0001)	24.5 ± 1.1 (q<0.0001)	28.1 ± 0.3 (q<0.0001)	3.3 ± 0.5 (ns)	42.10
siCDCA8 -01	6261	10.6 ± 0.4 (q<0.0001)	7.9 ± 0.8 (q<0.001)	26.6 ± 0.6 (q<0.0001)	43.2 ± 0.5 (q<0.0001)	82.17
siCDCA8 -02	5595	10.4 ± 0.7 (q<0.0001)	8.7 ± 0.3 (ns)	23.8 ± 1.1 (q<0.0001)	45.2 ± 1.5 (q<0.0001)	60.19
siCDCA8 -03	5073	30.5 ± 1.0 (q<0.0001)	14.6 ± 0.9 (q<0.0001)	27.0 ± 0.8 (q<0.0001)	13.6 ± 1.3 (q<0.0001)	23.40
siCDCA8 -04	8186	23.7 ± 0.4	12.0 ± 0.8	28.7 ± 0.5	20.4 ± 0.1	44.40

		(q<0.0001)	(q<0.01)	(q<0.0001)	(q<0.0001)	
siCIAO2B -01	16932	42.3 ± 2.1 (q<0.0001)	32.0 ± 4.2 (q<0.0001)	16.3 ± 0.8 (q<0.0001)	1.7 ± 0.0 (q<0.001)	24.41
siCIAO2B -03	14686	38.2 ± 1.6 (q<0.0001)	32.7 ± 1.7 (q<0.0001)	17.1 ± 0.7 (q<0.0001)	2.1 ± 0.2 (ns)	7.62
siCIAO2B -04	19264	41.6 ± 1.5 (q<0.0001)	24.0 ± 1.8 (q<0.0001)	21.9 ± 0.8 (q<0.001)	4.8 ± 0.2 (q<0.0001)	15.48
siCIAO2B -18	14660	41.3 ± 3.7 (q<0.0001)	37.7 ± 3.5 (q<0.0001)	10.1 ± 0.3 (q<0.0001)	0.9 ± 0.1 (q<0.0001)	26.18
siDYNC1H1 -01	15255	32.0 ± 0.3 (q<0.0001)	25.3 ± 0.9 (q<0.0001)	33.1 ± 1.0 (q<0.0001)	4.0 ± 0.7 (q<0.001)	22.40
siDYNC1H1 -02	16855	33.3 ± 0.2 (q<0.0001)	21.1 ± 0.8 (q<0.0001)	26.3 ± 0.2 (q<0.0001)	7.1 ± 0.6 (q<0.0001)	24.91
siDYNC1H1 -03	10371	23.0 ± 0.7 (q<0.0001)	22.4 ± 1.1 (q<0.0001)	39.2 ± 0.1 (q<0.0001)	6.9 ± 0.4 (q<0.0001)	79.29
siDYNC1H1 -04	21012	37.7 ± 1.3 (q<0.0001)	18.7 ± 1.3 (q<0.0001)	23.3 ± 0.5 (q<0.0001)	7.0 ± 0.3 (q<0.0001)	9.05
siENY2 -01	18169	39.6 ± 0.7	25.0 ± 0.8	19.3 ± 0.3	5.3 ± 0.1	15.62

		(q<0.0001)	(q<0.0001)	(q<0.01)	(q<0.0001)	
siENY2 -02	10196	43.1 ± 1.4 (q<0.0001)	15.5 ± 0.7 (q<0.0001)	26.4 ± 1.3 (q<0.0001)	7.0 ± 0.9 (q<0.0001)	29.06
siENY2 -03	21547	35.8 ± 0.5 (q<0.0001)	24.5 ± 0.9 (q<0.0001)	21.2 ± 0.9 (ns)	5.7 ± 0.7 (q<0.0001)	24.64
siENY2 -04	14896	43.8 ± 1.6 (q<0.0001)	28.6 ± 0.9 (q<0.0001)	16.5 ± 0.4 (q<0.0001)	2.1 ± 0.5 (ns)	16.54
siFAM98A -01	7481	38.5 ± 1.5 (q<0.0001)	24.4 ± 1.0 (q<0.0001)	23.8 ± 0.5 (q<0.0001)	3.1 ± 0.4 (ns)	23.43
siFAM98A -02	9898	31.0 ± 3.6 (q<0.0001)	32.0 ± 1.7 (q<0.0001)	23.9 ± 1.2 (q<0.0001)	5.1 ± 0.6 (q<0.0001)	30.61
siFAM98A -03	31999	47.9 ± 0.8 (ns)	11.2 ± 0.6 (ns)	20.0 ± 0.4 (ns)	3.6 ± 0.2 (q<0.0001)	-1.10
siFAM98A -04	12092	33.1 ± 3.6 (q<0.0001)	35.2 ± 3.1 (q<0.0001)	18.8 ± 0.7 (q<0.001)	4.1 ± 0.4 (q<0.0001)	37.85
siH1-10 -01	19495	40.2 ± 7.0 (q<0.0001)	17.5 ± 5.7 (q<0.0001)	27.4 ± 1.0 (q<0.0001)	5.4 ± 0.3 (q<0.0001)	19.98
siH1-10 -02	3095	26.7 ± 1.4	10.9 ± 1.3	37.6 ± 4.7	5.7 ± 0.4	38.30

		(q<0.0001)	(ns)	(q<0.0001)	(q<0.0001)	
siH1-10 -03	20063	54.2 ± 0.6 (q<0.0001)	10.3 ± 1.4 (ns)	19.8 ± 0.7 (ns)	2.1 ± 0.2 (ns)	16.89
siH1-10 -04	5378	64.3 ± 5.4 (q<0.0001)	16.2 ± 5.3 (q<0.0001)	9.0 ± 0.6 (q<0.0001)	1.5 ± 0.5 (q<0.001)	10.30
siINCENP -01	5191	14.1 ± 0.5 (q<0.0001)	9.3 ± 1.0 (ns)	29.6 ± 0.7 (q<0.0001)	28.0 ± 2.1 (q<0.0001)	63.31
siINCENP -02	6073	13.1 ± 1.2 (q<0.0001)	7.9 ± 0.3 (q<0.001)	43.1 ± 2.0 (q<0.0001)	25.7 ± 0.9 (q<0.0001)	107.86
siINCENP -03	5151	14.6 ± 1.9 (q<0.0001)	9.5 ± 0.3 (ns)	42.4 ± 2.7 (q<0.0001)	19.4 ± 0.9 (q<0.0001)	84.39
siINCENP -04	7989	19.9 ± 0.6 (q<0.0001)	9.1 ± 0.3 (ns)	38.4 ± 0.4 (q<0.0001)	20.0 ± 1.7 (q<0.0001)	65.05
siINKA1 -01	8245	24.3 ± 0.6 (q<0.0001)	13.0 ± 1.4 (q<0.0001)	50.4 ± 0.5 (q<0.0001)	5.7 ± 0.2 (q<0.0001)	81.39
siINKA1 -02	18874	33.1 ± 0.5 (q<0.0001)	15.1 ± 0.7 (q<0.0001)	28.0 ± 1.2 (q<0.0001)	10.6 ± 0.9 (q<0.0001)	12.47
siINKA1 -03	20994	56.5 ± 1.3	21.2 ± 1.2	14.5 ± 0.1	1.3 ± 0.2	17.64

		(q<0.0001)	(q<0.0001)	(q<0.0001)	(q<0.0001)	
siINKA1 -04	7543	30.0 ± 3.2 (q<0.0001)	16.8 ± 2.7 (q<0.0001)	38.3 ± 1.2 (q<0.0001)	6.9 ± 0.2 (q<0.0001)	31.55
siKTN1 -17	15844	43.6 ± 1.3 (q<0.0001)	26.6 ± 1.7 (q<0.0001)	16.9 ± 0.8 (q<0.0001)	3.5 ± 0.4 (q<0.01)	41.87
siKTN1 -18	2762	22.7 ± 2.1 (q<0.0001)	10.0 ± 0.7 (ns)	47.8 ± 1.8 (q<0.0001)	4.2 ± 0.7 (q<0.0001)	44.02
siKTN1 -19	29473	55.4 ± 2.1 (q<0.0001)	10.9 ± 0.7 (ns)	19.2 ± 0.9 (ns)	2.7 ± 0.3 (ns)	4.61
siKTN1 -20	22507	46.0 ± 0.4 (ns)	18.1 ± 0.2 (q<0.0001)	22.4 ± 0.6 (q<0.0001)	3.9 ± 0.2 (q<0.001)	16.93
siLUC7L -01	1855	30.5 ± 2.8 (q<0.0001)	11.6 ± 1.4 (q<0.01)	34.1 ± 3.0 (q<0.0001)	6.2 ± 2.2 (q<0.0001)	36.19
siLUC7L -02	9450	39.6 ± 2.7 (q<0.0001)	30.3 ± 3.8 (q<0.0001)	15.7 ± 0.2 (q<0.0001)	2.4 ± 0.6 (ns)	6.67
siLUC7L -03	24023	51.5 ± 0.7 (q<0.01)	25.4 ± 1.1 (q<0.0001)	14.2 ± 0.6 (q<0.0001)	1.6 ± 0.2 (q<0.001)	7.84
siLUC7L -04	15445	38.4 ± 1.5	28.5 ± 2.5	21.1 ± 0.7	2.2 ± 0.0	22.01

		(q<0.0001)	(q<0.0001)	(q<0.01)	(ns)	
siMDN1 -03	22383	61.8 ± 4.5 (q<0.0001)	17.6 ± 4.3 (ns)	11.6 ± 0.5 (q<0.0001)	1.5 ± 0.2 (q<0.001)	16.23
siMDN1 -17	10682	64.0 ± 0.2 (q<0.0001)	10.7 ± 0.9 (ns)	16.0 ± 0.3 (q<0.0001)	1.4 ± 0.1 (q<0.0001)	10.86
siMDN1 -18	18302	52.2 ± 0.9 (q<0.001)	24.3 ± 3.0 (q<0.0001)	14.6 ± 0.6 (q<0.0001)	1.4 ± 0.2 (q<0.001)	8.92
siMDN1 -19	16924	51.6 ± 1.8 (q<0.01)	15.7 ± 1.3 (q<0.0001)	18.9 ± 1.2 (q<0.001)	2.1 ± 0.2 (ns)	28.03
siRACGAP1 -01	10345	28.6 ± 1.8 (q<0.0001)	19.5 ± 1.6 (q<0.0001)	31.1 ± 0.8 (q<0.0001)	10.7 ± 0.9 (q<0.0001)	51.56
siRACGAP1 -02	14099	33.4 ± 1.3 (q<0.0001)	15.0 ± 0.3 (q<0.0001)	27.8 ± 0.6 (q<0.0001)	9.0 ± 1.3 (q<0.0001)	34.13
siRACGAP1 -03	9246	20.7 ± 2.0 (q<0.0001)	14.2 ± 0.6 (q<0.0001)	37.3 ± 1.6 (q<0.0001)	17.2 ± 1.5 (q<0.0001)	66.49
siRACGAP1 -04	6630	15.1 ± 1.7 (q<0.0001)	15.7 ± 1.0 (q<0.0001)	34.3 ± 0.7 (q<0.0001)	23.8 ± 2.4 (q<0.0001)	58.42
siRFC1 -01	22242	36.7 ± 1.0	21.5 ± 1.7	28.7 ± 0.9	5.1 ± 0.2	12.31

		(q<0.0001)	(q<0.0001)	(q<0.0001)	(q<0.0001)	
siRFC1 -02	18739	35.2 ± 2.3 (q<0.0001)	24.5 ± 0.3 (q<0.0001)	27.6 ± 0.9 (q<0.0001)	3.7 ± 0.4 (q<0.01)	15.79
siRFC1 -03	18566	38.5 ± 2.1 (q<0.0001)	24.6 ± 1.4 (q<0.0001)	26.9 ± 0.6 (q<0.0001)	2.6 ± 0.2 (ns)	20.99
siRFC1 -04	32219	46.9 ± 0.6 (ns)	10.8 ± 1.0 (ns)	21.8 ± 0.2 (q<0.001)	4.5 ± 0.1 (q<0.0001)	0.60
siSTK24 -05	13758	41.3 ± 2.1 (q<0.0001)	34.8 ± 1.9 (q<0.0001)	15.0 ± 0.6 (q<0.0001)	2.0 ± 0.3 (q<0.01)	7.04
siSTK24 -21	30865	52.6 ± 2.3 (q<0.0001)	11.5 ± 1.7 (ns)	19.3 ± 0.4 (q<0.01)	2.8 ± 0.2 (ns)	1.81
siSTK24 -22	15617	37.4 ± 3.9 (q<0.0001)	25.7 ± 2.9 (q<0.0001)	23.6 ± 0.6 (q<0.0001)	3.6 ± 0.4 (q<0.01)	17.66
siSTK24 -23	11330	43.4 ± 3.4 (q<0.0001)	37.5 ± 2.9 (q<0.0001)	12.0 ± 0.8 (q<0.0001)	1.4 ± 0.1 (q<0.0001)	29.89
siTPX2 -01	3189	18.7 ± 2.6 (q<0.0001)	12.4 ± 2.2 (q<0.001)	49.8 ± 2.8 (q<0.0001)	9.0 ± 0.3 (q<0.0001)	38.82
siTPX2 -02	6023	16.5 ± 1.3	10.6 ± 1.1	50.9 ± 0.6	6.7 ± 0.7	102.59

		(q<0.0001)	(ns)	(q<0.0001)	(q<0.0001)	
siTPX2 -03	10750	47.2 ± 3.5 (ns)	22.9 ± 2.6 (q<0.0001)	15.9 ± 0.4 (q<0.0001)	2.8 ± 0.4 (ns)	39.05
siTPX2 -04	7667	34.5 ± 2.3 (q<0.0001)	18.3 ± 0.6 (ns)	26.0 ± 1.3 (q<0.0001)	6.1 ± 0.9 (q<0.0001)	31.55
siWRAP53 -19	31592	49.2 ± 1.4 (ns)	12.6 ± 1.0 (q<0.001)	21.4 ± 0.9 (q<0.01)	4.2 ± 0.4 (q<0.0001)	2.90
siWRAP53 -20	17584	38.1 ± 1.9 (q<0.0001)	33.2 ± 0.6 (q<0.0001)	16.5 ± 1.4 (q<0.0001)	2.4 ± 0.3 (ns)	28.11
siWRAP53 -21	18888	41.2 ± 0.6 (q<0.0001)	26.9 ± 0.5 (q<0.0001)	20.9 ± 0.7 (ns)	3.3 ± 0.3 (ns)	18.45
siWRAP53 -22	17244	41.0 ± 1.0 (q<0.0001)	29.8 ± 0.9 (q<0.0001)	18.6 ± 0.2 (q<0.001)	2.4 ± 0.2 (ns)	38.53
siXRCC5 -01	24899	50.9 ± 2.0 (q<0.01)	21.5 ± 1.4 (q<0.0001)	18.8 ± 0.5 (q<0.01)	1.9 ± 0.3 (q<0.01)	8.08
siXRCC5 -02	25424	49.4 ± 1.2 (ns)	13.2 ± 0.8 (q<0.0001)	23.2 ± 1.0 (q<0.0001)	3.3 ± 0.3 (ns)	6.89
siXRCC5 -03	24903	48.2 ± 2.0	15.0 ± 0.7	25.8 ± 0.8	2.2 ± 0.4	-1.50



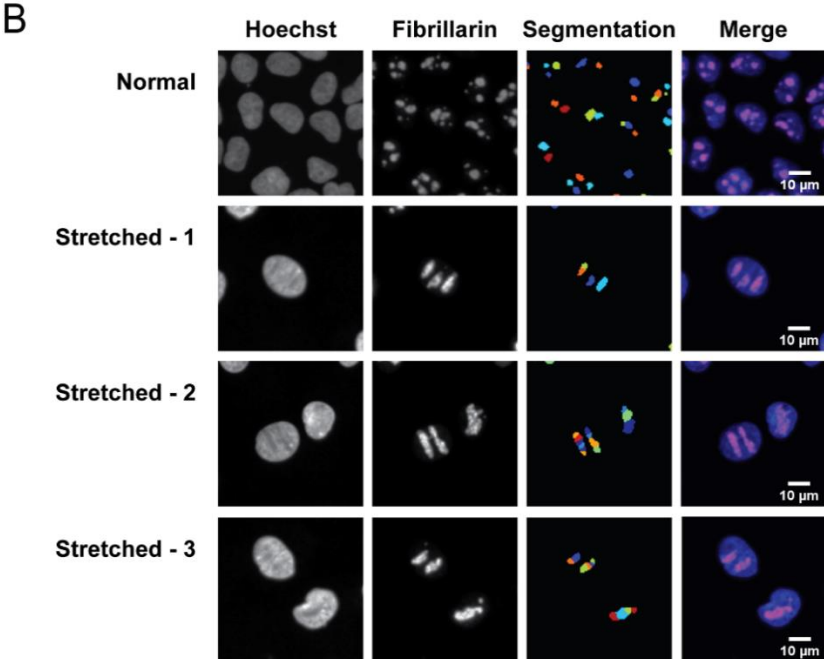
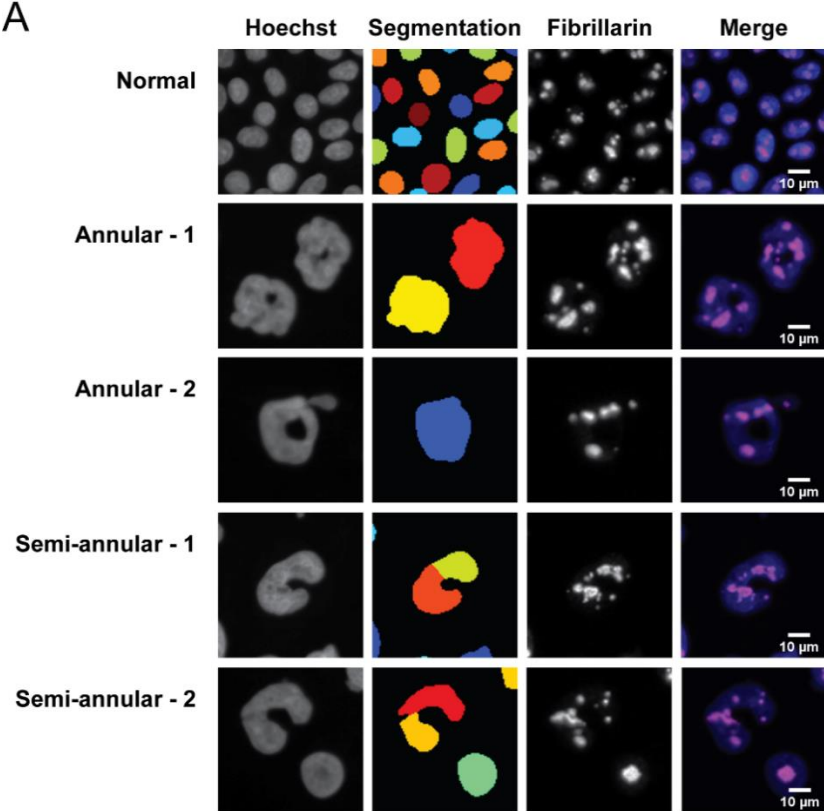
		(ns)	(q<0.0001)	(q<0.0001)	(ns)	
siXRCC5 -04	31137	53.9 ± 0.8	10.2 ± 0.3	19.3 ± 0.2	2.7 ± 0.2	-2.68
		(ns)	(ns)	(q<0.01)	(ns)	

**Supplemental Table S5.** Normalized percent effect (NPE) for each siRNA in the pool of 4 comparing NPE for all cells to cells in G2/M only.

Hits	All cells				G2/M cells			
	1	2	3	4	1	2	3	4
siKIF11	89.98	118.62	74.75	116.70	104.40	130.50	111.60	133.20
siINCENP	63.31	107.86	84.39	65.05	103.10	119.60	106.60	85.90
siCDCA8	82.17	60.19	23.40	44.40	116.70	90.80	41.80	74.10
siRACGAP1	51.56	34.13	66.49	58.42	73.40	58.90	89.90	71.90
siINKA1	81.39	12.47	17.64	71.44	91.90	18.70	48.80	91.60
siTPX2	38.82	102.59	39.05	31.55	44.60	125.30	91.20	64.60
siKTN1	41.87	44.02	4.61	16.93	81.80	51.40	14.90	31.90
siFAM98A	23.43	30.61	-1.10	37.85	47.70	51.00	-2.20	64.00
siABCE1	50.71	22.63	26.31	9.86	68.80	37.60	60.00	16.50
siDYNC1H1	22.40	24.91	79.29	9.05	25.70	41.80	104.80	20.90
siWRAP53	2.90	28.11	18.45	38.53	7.50	53.00	35.60	74.90
siATAD5	55.60	16.49	25.01	22.60	80.00	30.30	40.90	23.70
siCCN4	17.85	18.51	23.89	42.10	53.00	30.50	32.30	63.10
siENY2	15.62	29.06	24.64	16.54	32.70	38.70	49.70	44.00
siCIAO2B	24.41	7.62	15.48	26.18	55.40	24.40	28.10	64.20
siH1-10	19.98	38.30	16.89	10.30	26.20	48.60	32.10	37.80
siLUC7L	36.19	6.67	7.84	22.01	43.50	18.50	29.50	46.80
siRFC1	12.31	15.79	20.99	0.60	13.60	20.00	26.80	3.50
siMDN1	16.23	10.86	8.92	28.03	50.30	30.80	21.50	53.00
siSTK24	7.04	1.81	17.66	29.89	23.10	7.50	35.80	54.50
siXRCC5	8.08	6.89	-1.50	-2.68	17.10	11.20	-8.10	-5.90

Supplemental Figures and Figure Legends

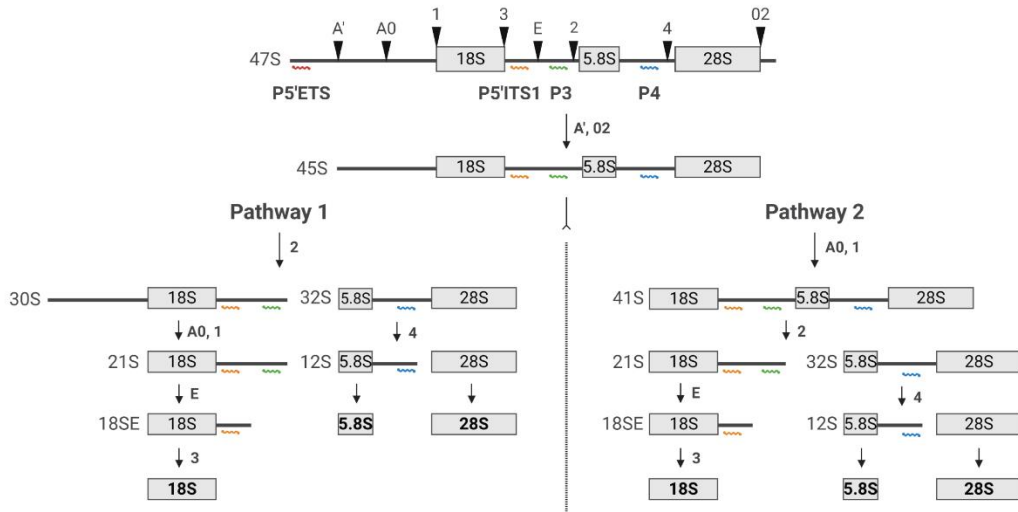
Supplemental Figure S1



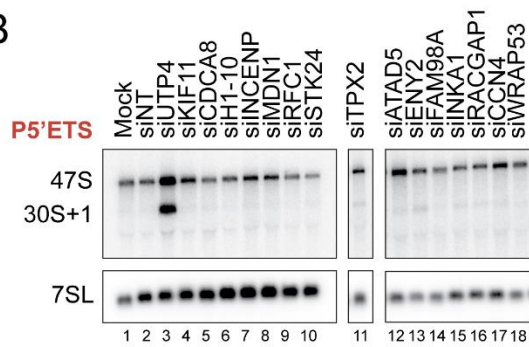
**Supplemental Figure S1.** Analysis of CellProfiler pipeline identification of atypical nuclei and nucleoli reveals poor segmentation in some cases. In an observational analysis, we looked at a subset of screen images where atypical nuclei and nucleoli were observed. (A) Pipeline segmentation of annular and semi-annular nuclei revealed inconsistent nuclear identification. Annular nuclei were sometimes segmented as a single nucleus, whereas semi-annular nuclei were sometimes segmented as more than one nucleus. Each colored spot represents a single segmented nucleus. In the merge, blue=Hoechst (nuclei) and pink=fibrillarin (nucleoli). Normal nuclei shown were from siRISC-free and atypical nuclei shown were from siINCENP. (B) Pipeline segmentation of “stretched” nucleoli were sometimes incorrectly identified as multiple nucleoli. Each colored spot represents a single segmented nucleolus. In the merge, blue=Hoechst (nuclei) and pink=fibrillarin (nucleoli). Normal nucleoli shown were from siRISC-free and atypical nucleoli shown were from siMDN1.

# Supplemental Figure S2

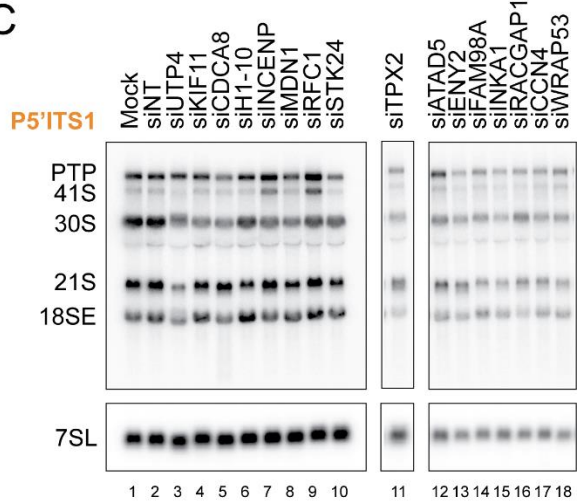
**A**



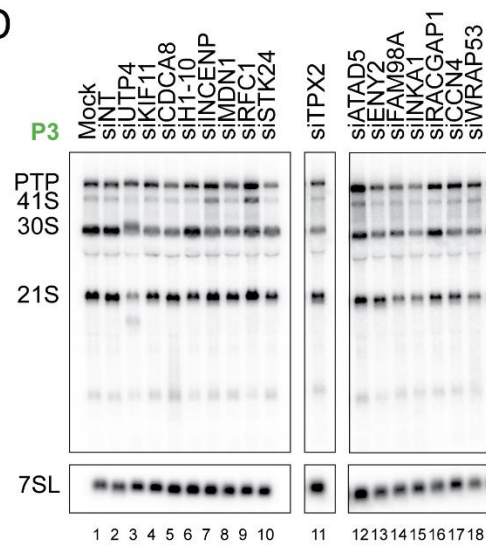
**B**



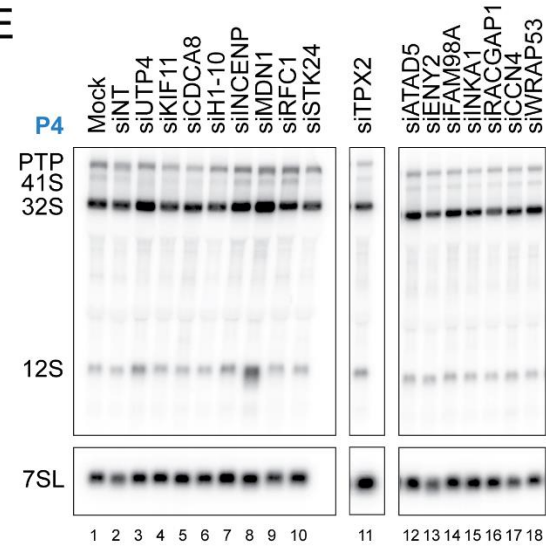
**C**



**D**

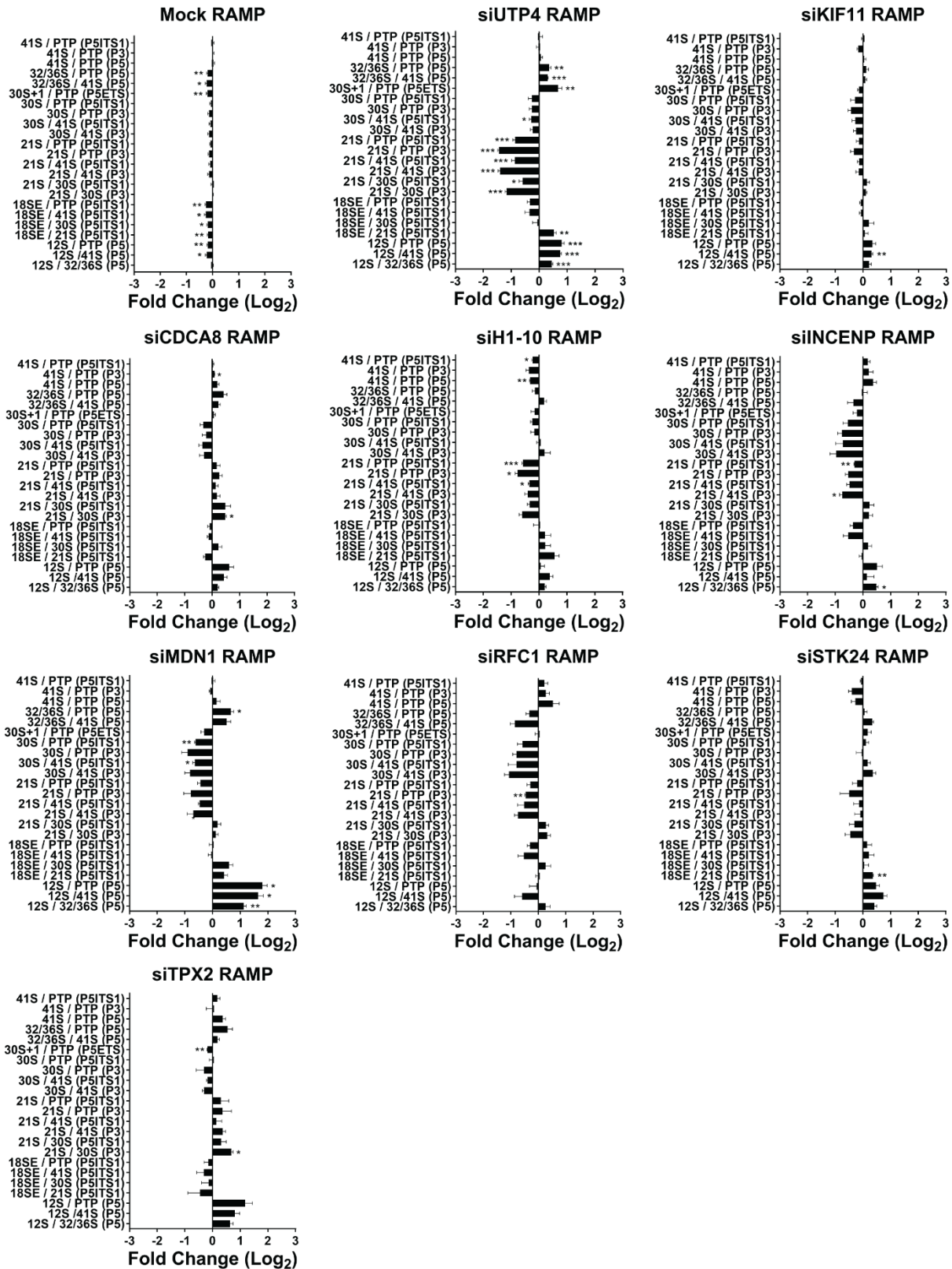


**E**



**Supplemental Figure S2.** Qualitative analysis of pre-rRNA intermediates reveals no obvious pre-rRNA processing defects. Depletion of a subset of nucleolar (n=7) and non-nucleolar (n=7) hits in MCF10A cells were tested by northern blot to ask whether depletion affects steady-state levels of pre-rRNA intermediates, and whether pre-rRNA processing defects can be inferred by observed changes in precursor-product relationships. (A) Pre-rRNA processing diagram labeled with the oligonucleotide probes used to detect pre-rRNA intermediates. The two predominant pathways for releasing the mature ribosomal RNAs (18S, 5.8S, and 28S) from the primary 47S transcript are depicted. Cleavage sites are indicated with black triangles and are listed next to the black arrows. (B) Representative northern blots using probe P5'ETS. (C) Representative northern blots using probe P5'ITS1. (D) Representative northern blots using probe P3. (E) Representative northern blot using probe P4. In each blot, mock and siNT were included as negative controls, and siUTP4 was included as a positive control. PTP=primary transcript plus, 43S-47S.

# Supplemental Figure S3



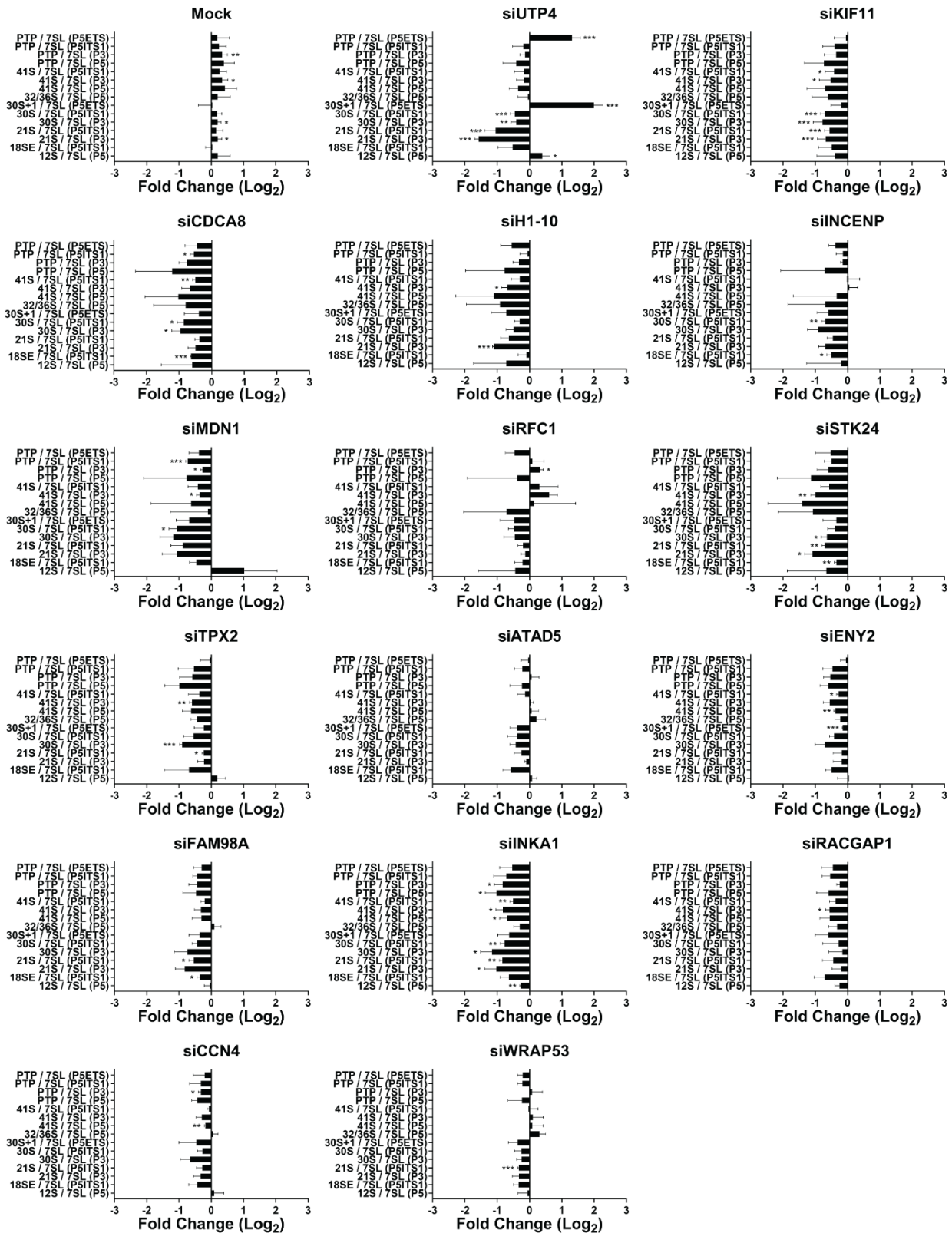
**Supplemental Figure S3.** Quantitative analysis of the northern blots of nucleolar hits reveals MDN1-depletion significantly affects processing of the 12S pre-rRNA. Quantitative analysis of the northern blots of nucleolar hits in Supplemental Figure S2 was performed by Ratio Analysis of Multiple Precursors (RAMP) relative to siNT (mean  $\pm$  SD). Mock-treated cells and UTP4-depleted cells were included as negative and positive controls, respectively. Depletion of the screen positive control, KIF11, was also analyzed. Statistical significance was calculated for 3 replicates by unpaired t tests for each ratio with the Holm-Sidak method of correction for multiple comparisons (\*=adjusted  $p < 0.05$ , \*\*=adjusted  $p < 0.01$ , \*\*\*=adjusted  $p < 0.001$ ;  $n=3$ ). PTP=primary transcript plus, 43S-47S.





**Supplemental Figure S4.** Quantitative analysis of the northern blots of non-nucleolar hits reveals pre-rRNA processing is largely unaffected. Quantitative analysis of the northern blots of non-nucleolar hits in Supplemental Figure S2 was performed by Ratio Analysis of Multiple Precursors (RAMP) relative to siNT (mean  $\pm$  SD). Statistical significance was calculated for 3 replicates by unpaired t tests for each ratio with the Holm-Sidak method of correction for multiple comparisons (\*=adjusted  $p < 0.05$ , \*\*=adjusted  $p < 0.01$ , \*\*\*=adjusted  $p < 0.001$ ;  $n=3$ ). PTP=primary transcript plus, 43S-47S.

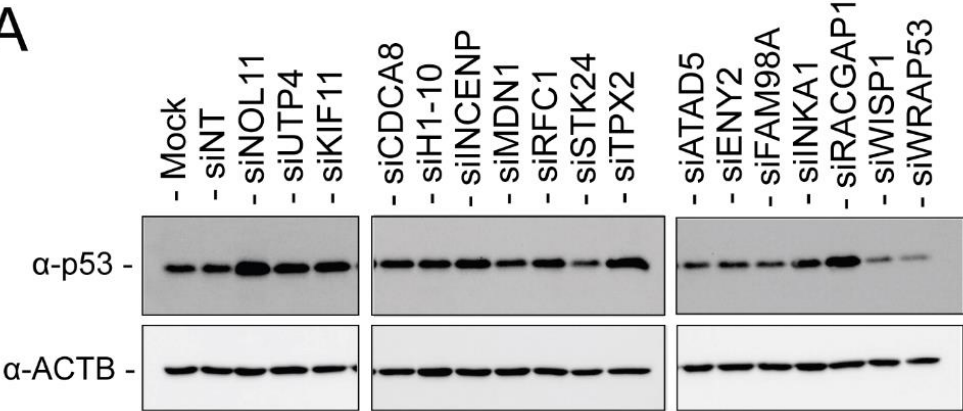
# Supplemental Figure S5



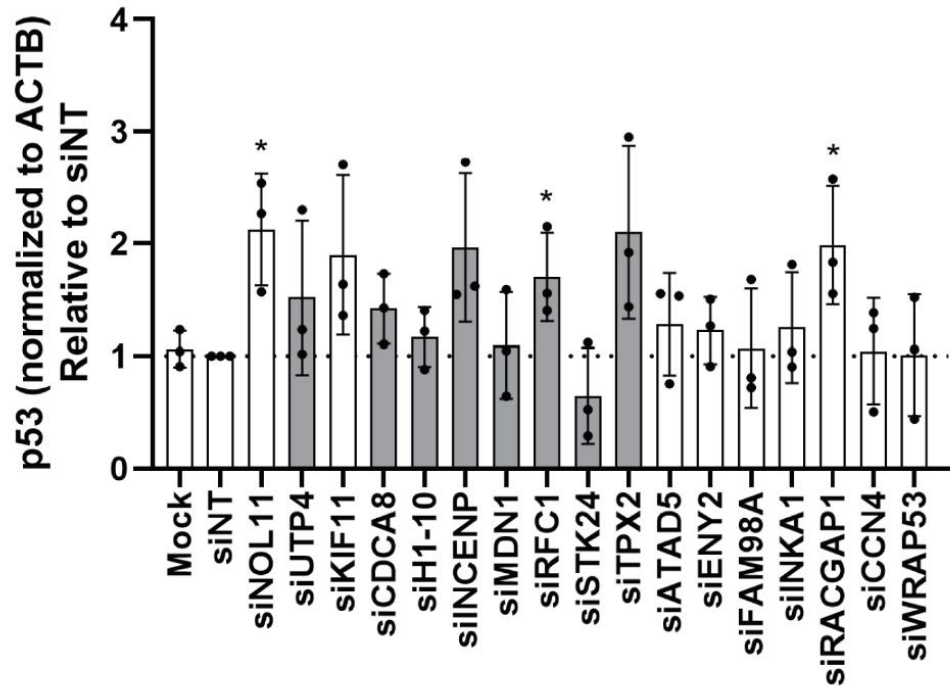
**Supplemental Figure S5.** Quantitative analysis of pre-rRNA intermediates relative to the 7SL RNA loading control reveals a trending, but non-significant, decrease in levels of pre-rRNA intermediates. Quantitative analysis of the northern blots in Supplemental Figure S2 was performed relative to the RNA component of the signal recognition particle, 7SL, relative to siNT (mean  $\pm$  SD). Statistical significance for 3 replicates was calculated by unpaired t tests for each ratio with the Holm-Sidak method of correction for multiple comparisons (\*=adjusted  $p < 0.05$ , \*\*=adjusted  $p < 0.01$ , \*\*\*=adjusted  $p < 0.001$ ;  $n=3$ ). PTP=primary transcript plus, 43S-47S.

Supplemental Figure S6

A



B



**Supplemental Figure S6.** Depletion of only 2 hits results in the stabilization of the tumor suppressor protein, p53. (A) Representative western blots from the total protein harvested from MCF10A cells depleted of the screen hits in Figure 7 and Table 1 (in bold). Protein was quantified by Bradford assay and run on a 10% SDS-PAGE gel followed by western blots using an HRP-conjugated antibody to the tumor suppressor protein, p53 (TP53). Beta-actin (ACTB) was used as a loading control. Mock and siNT-treated cells=negative controls. siNOL11=positive control. siUTP4 was included as a known ribosome biogenesis factor, and siKIF11 was included because it was the screen positive control and elicited a RNAPI and protein synthesis defect upon treatment. (B) Quantification of results in A from 3 replicates. ImageJ was used to quantify the differences in p53 signal intensity, normalized to the beta-actin signal intensity. Statistical significance for the 3 replicates relative to siNT was calculated by two-tailed, unpaired t tests ( $*=p<0.05$ ;  $n=3$ ). Data are shown as a bar graph (mean  $\pm$  SD), and with each replicate represented as a dot. Gray=nucleolar proteins; white=non-nucleolar proteins.