Supplementary Methods

DR-GFP and gene targeting HR assays

HR assays were performed as described previously (Pierce et al., 1999). Briefly, we used a 293 cell line (293/DR-GFP) stably expressing a mutated *GFP* gene (*SceGFP*) that had an insert containing in-frame stop codons and the 18-bp I-SceI recognition sequence. The DR-GFP repair substrate also contained an internal fragment of *GFP* (*truncGFP*) down-stream of *SceGFP* (Pierce et al., 1999). Expression of neither *SceGFP* nor *truncGFP* could yield a functional GFP protein. To assay the rate of HR, 293/DR-GFP cells were transfected with an I-SceI-encoding plasmid along with LACZ or MMS21 siRNA oligonucleotides. To determine the transfection efficiency, 293/DR-GFP cells were transfected with a GFP-expression plasmid and either LACZ or MMS21 siRNA oligonucleotides. The cells were then grown for 3 days. The percentage of HR was determined by measuring the percentage of GFP-positive cells using a FACScan flow cytometer (BD Biosciences) and normalizing to the transfection efficiency for each condition.

Gene targeting assays were performed essentially as described previously (Porteus and Baltimore, 2003). Briefly, we used a 293 cell line (293/A658) stably expressing a *GFP* gene that had an insert containing in-frame stop codons and a I-SceI recognition site (5'-TAGGATAACAGGGTAAT-3') at bp 327 of the *GFP* coding region. The expression of GFP was driven by a hybrid CMV/CBA promoter. This mutant *GFP* served as the artificial chromosomal gene target. The I-SceI/repair plasmid (A979) contained two cassettes: the first is an I-SceI expression cassette using the CMV/CBA promoter; the second is a 2100 bp repair substrate that contains a truncated *GFP* gene (*truncGFP*) followed by an additional 1300 bp of 3'-homology to the mutated *GFP* genomic target. Gene targeting was measured by transfecting

293/A658 cells with the I-SceI/repair plasmid and the indicated siRNA oligonucleotides. To normalize for differences in transfection efficiency, 293/A658 cells were also transfected with a GFP-expression plasmid and the indicated siRNAs. The cells were then grown for 3 days. The percentage of GFP-positive cells was measured by a FACScan flow cytometer and normalized to the transfection efficiency for each condition to give the gene targeting efficiency.

Ligation-mediated quantitative PCR (LM-QPCR)

293/A658D cells were transfected with siRNAs toward LACZ or hMMS21. Twenty-four hours after siRNA transfection, cells were transfected with a CMV/CBA I-SceI expression construct. Genomic DNA was collected, 0, 24, or 48 hrs after transfection with a DNAeasy extraction kit (Qiagen) per manufacture's instructions. I-SceI-induced 3' overhangs at DSBs were converted to blunt ends by treatment of 5 μg genomic DNA with T4 DNA polymerase (New England Biolabs) for 15 min at 12 ºC. The resulting blunt-ended genomic DNA was purified using a QIAquick spin column (Qiagen). The asymmetric annealed BW linker oligonucleotides (BW-1 and BW-2) were then ligated to the blunt-ended genomic DNA at 16 ºC for 16 hrs as described previously (Curry et al., 2005). The resulting linker-ligated genomic DNA was purified using a QIAquick spin column. The presence of linker-ligated DSBs in the genomic DNA was assayed by QPCR with 2X iTaq (Bio-Rad Laboratories) on an ABI-7500 (Applied Biosystems) using primers spanning the linker region (linker #2) and *GFP* (*GFP* #3). Results were normalized for amount of genomic DNA in the samples using primers toward *GFP* only (*GFP* #5). The primer sequences used are listed in Table S1.

End-joining assay

We used a 293 cell line (293/1040) stably expressing an end-joining reporter (Figure 3A) that contained a *GFP* gene flanked by I-SceI recognition sites driven by a CMV/CBA promoter for assaying end-joining. The *CD8α* gene was located downstream of the *GFP* gene and was not constitutively expressed due to the lack of an IRES. 293/1040 cells were transfected with the indicated siRNAs and either an I-SceI expression plasmid or an RFP expression plasmid as a transfection efficiency control. If I-SceI cuts both sites flanking *GFP* and end-joining occurs, GFP expression would be lost and CD8 expression would be gained. CD8 expression was measured by staining with phycoerytherin-conjugated anti-CD8 monoclonal antibody (Ditech). Cells were analyzed for the loss of GFP expression and gain of CD8 expression by FACS 3-5 days after transfection. The end-joining rate was determined by counting the percentage of GFP-CD8+ cells and normalizing to the transfection efficiency.

Sister-chromatid exchange (SCE) assay

293/A658 cells were transfected with the indicated siRNAs for 24 hrs. The cells were replated and incubated in the presence of 10 μM BrdU for 40 hrs (two cell divisions). Where indicated, 2.5 nM camptothecin was added during the BrdU labeling to induce a greater number of SCEs. Colcemid (150 ng/mL) was added during the final 30 minutes to enrich for mitotic cells. Cells were collected by trypsinization and washed in PBS. Cells were swelled in 75 mM KCl for 16 min at 37ºC, followed by centrifugation. Cell pellets were resuspended in fixative (3:1 solution of methanol:glacial acetic acid) and incubated for 20 min at 4ºC. Cells were washed in fixative two more times. After the final wash, cells were resuspended in fixative and dropped onto cold slides. Slides were allowed to air dry in the dark for 2-3 days. Chromosomes were then differentially stained for 5 min with 0.1 mg/mL acridine orange (Molecular Probes). Slides were

washed extensively for 2 min under running water followed by 1-min incubation and mounting in Sorenson Buffer, pH 6.8 (0.1M Na_2HPO_4, 0.1M NaH_2PO_4). Slides were immediately viewed with a 63x objective on a Zeiss Axiovert 200M fluorescence microscope. Images were acquired with a CCD camera using Slidebook imaging software (Intelligent Imaging Innovations). Images were analyzed for the number of SCE by counting the number of crossover events per metaphase. A minimum of 50 metaphases were scored for each experiment over three independent experiments.

Long tract sister-chromatid recombination (LTGC/SCR) assay

LTGC/SCR assays using the U2OS/SCR cells (kindly provided by Dr. Ralph Scully, Boston, MA) were essentially described previously (Puget et al., 2005). Briefly, U2OS/SCR cells were transfected with the indicated siRNAs for 24 hrs and subsequently transfected with an I-SceI expression plasmid for 24 additional hours. Cells were then collected and replated at 50,000, 100,000, and 200,000 cells per 10cm^2 dish in duplicates. Cells were incubated for four days before addition of blasticidin (Invitrogen, 5 μg/mL). Fourteen days after continual selection, blasticidin resistant (BsdR+) cells were fixed and stained with crystal violet before counting. The percentage of cells undergone LTGC/SCR was normalized to the plating and transfection efficiency of the LACZ-RNAi and MMS21-RNAi cells. Plating efficiency was determined by plating 200 cells per 60 cm^2 dish in quadruplicates after RNAi and I-SceI transfection. Cells were incubated as describe above except in the absence of blasticidin. Transfection efficiency was determined by transfection of GFP-expression plasmid followed by flow cytometry.

For determining the percentage of unequal, short tract gene conversion and intrachromatid recombination, we treated U2OS/SCR cells with RNAi for 24 hrs followed by

transfection of I-SceI. Cells were analyzed four days later by flow cytometry for the number of GFP-positive cells. Results were normalized to transfection efficiencies.

Comet assay

Comet assays were performed according to manufacturer's protocol (Trevigen) and as previously described (Potts and Yu, 2005). Briefly, cells were collected after the indicated treatments, mixed with low melt agarose and allowed to harden on slides. Cells were then incubated in lysis buffer followed by incubation in alkaline buffer. Slides were then washed in TBE and then placed in a horizontal electrophoresis apparatus and voltage was applied for 10 min at 12V. Slides were then immersed in 70% ethanol and then allowed to air dry at room temperature overnight. Slides were stained with SYBR Green 1 (Molecular Probes) for 10 min and then washed once in TBE. All incubations were performed in the dark to prevent unintended DNA damage. Slides were viewed using a 20X objective as described above. The percentage of cells displaying a comet tail was calculated. At least 100 cells were scored in each condition over three independent experiments.

Chromatin immunoprecipitation (ChIP) assay

293/A658 cells ($5x10^7$ cells per ChIP) were transfected with vector control or CMV/CBA I-SceI expression plasmid for 24 hrs. In experiments involving siRNA, cells were transfected with siRNAs for 24 hrs prior to transfection with the I-SceI expression plasmid. ChIP was performed as described previously (Aparicio et al., 2005). Cells were crosslinked in 0.37% formaldehyde and the reaction was quenched with 0.3 M glycine. The cells were washed in TBS and MC lysis buffer (10 mM Tris-Cl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, and 0.5% NP-40). The nuclear

pellet was then resuspended in sonication buffer (10 mM Tris-Cl, pH 7.5, 200 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 4% NP-40, 1% SDS, 3 mM PMSF, and 1X protease inhibitor cocktail (Roche)). Cells were sonicated at 60% duty cycle, four times for 30 sec continuously in an ice water bath with 2 min breaks between sonications. The fragmented chromatin was then cleared by centrifugation and the soluble fraction diluted 1:5 in FA lysis buffer (50 mM HEPES-KOH, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 2 mM PMSF, and 1X protease inhibitor cocktail). The diluted chromatin was then precleared with protein A-Sepharose beads (Bio-Rad). One percent of the resulting precleared chromatin was used as the input sample. To the remaining precleared chromatin, 2 µg of the indicated antibodies were added and incubated rotating overnight at 4ºC. The chromatin bound beads were washed in FA lysis buffer, ChIP wash buffer (10 mM Tris-Cl, pH 8.0, 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, and 0.5% sodium deoxycholate), and TE. Chromatin was then eluted from the beads by incubation in ChIP elution buffer (50 mM Tris-Cl, pH 7.5, 10 mM EDTA, and 1% SDS) at 65ºC for 10 min. The chromatin was then decrosslinked and proteins degraded. The DNA was then purified over a QIAquick spin column, per manufacturer's instructions. Conventional semi-quantitative PCR was performed on the resulting purified DNA after ChIP using with 25-30 cycles of amplification. Quantitative real-time PCR (QPCR) was performed as described above using validated primers toward the 5' side of the DSB (*GFP* #5) or the 3' side of the DSB (*GFP*#7) and *GAPDH* as a normalization control. The sequences of the primers used are listed in Table S1.

Supplementary Figures

Supplemental Figure 1 Knockdown of the hSMC5/6 or Cohesin Complexes Increases the Efficiency of Gene Targeting

(**A & B**) 293/A658 cells were transfected with the repair plasmid along with the indicated siRNA oligonucleotides and analyzed three days later by flow cytometry. GFP-positive cells are gated and shown in green. Experiments are performed in triplicates. Only one set of samples is shown.

(**C & D**) 293/A658 cells were transfected with the indicated siRNA and blotted with the indicated antibodies.

Supplemental Figure 2 I-SceI endonuclease activity is unaltered by hMMS21-RNAi.

(**A**) Schematic drawing of the integrated target in 293/A658 cells. The *GFP* gene contains an I-SceI recognition site followed by an XhoI recognition site. An XbaI recognition site is located 2.0kb downstream of the I-SceI recognition site. Upon I-SceI-induced DSB and digestion of genomic DNA with XbaI, a 2.0kB product is obtained that indicates I-SceI activity.

(**B**) 293/A658 cells were transfected with the indicated siRNAs. Twenty-four hours after RNAi, an I-SceI expression plasmid was transfected into the cells. Genomic DNA was collected at the indicated times after I-SceI transfection. Genomic DNA was digested with XbaI and southern blot analysis was performed to determine the amount of I-SceI-induced DSBs in the cells. A fragment of the A658 plasmid was used as the hybridization probe. As a control for I-SceI digestion, A658 plasmid containing the stop-I-SceI-*GFP* was digested with XbaI and XhoI to mimic an I-SceI-induced DSB. The 2.0kB I-SceI cut band is shown in the autoradiogram.

Quantitation of the relative intensities of the bands is shown. Image is a representative sample of multiple experiments.

(**C**) Ethidium bromide stained gel before southern blot hybridization, shown in (**B**), to illustrate the equal loading of DNA across samples. Image is a representative sample of multiple experiments.

Supplemental Figure 3 Knockdown of the hSMC5/6 or Cohesin Complexes Decreases the Number of Spontaneous SCEs

(**A**) 293/A658 cells transfected with the indicated siRNA for 24 hrs were labeled with 10 μM BrdU for 40 hrs (two cell divisions). Chromosome spreads were stained with acridine orange to distinguish sister chromosomes. Arrowheads indicate recombination events. Images are a representative sample of multiple experiments.

(**B**) Histogram of cells in (A) with the indicated number of sister-chromatid recombination events per cell. Results from three separate samples are averaged with the standard deviation indicated in the table to the right.

(**C**) Images of colony formation assays described and quantitated in Figure 4C and 4D. Images are a representative sample of multiple experiments.

Supplemental Figure 4 Gene Targeting Is Increased in Cells Arrested at G2/M

(**A**) FACS analysis of cells described in Figure 5A.

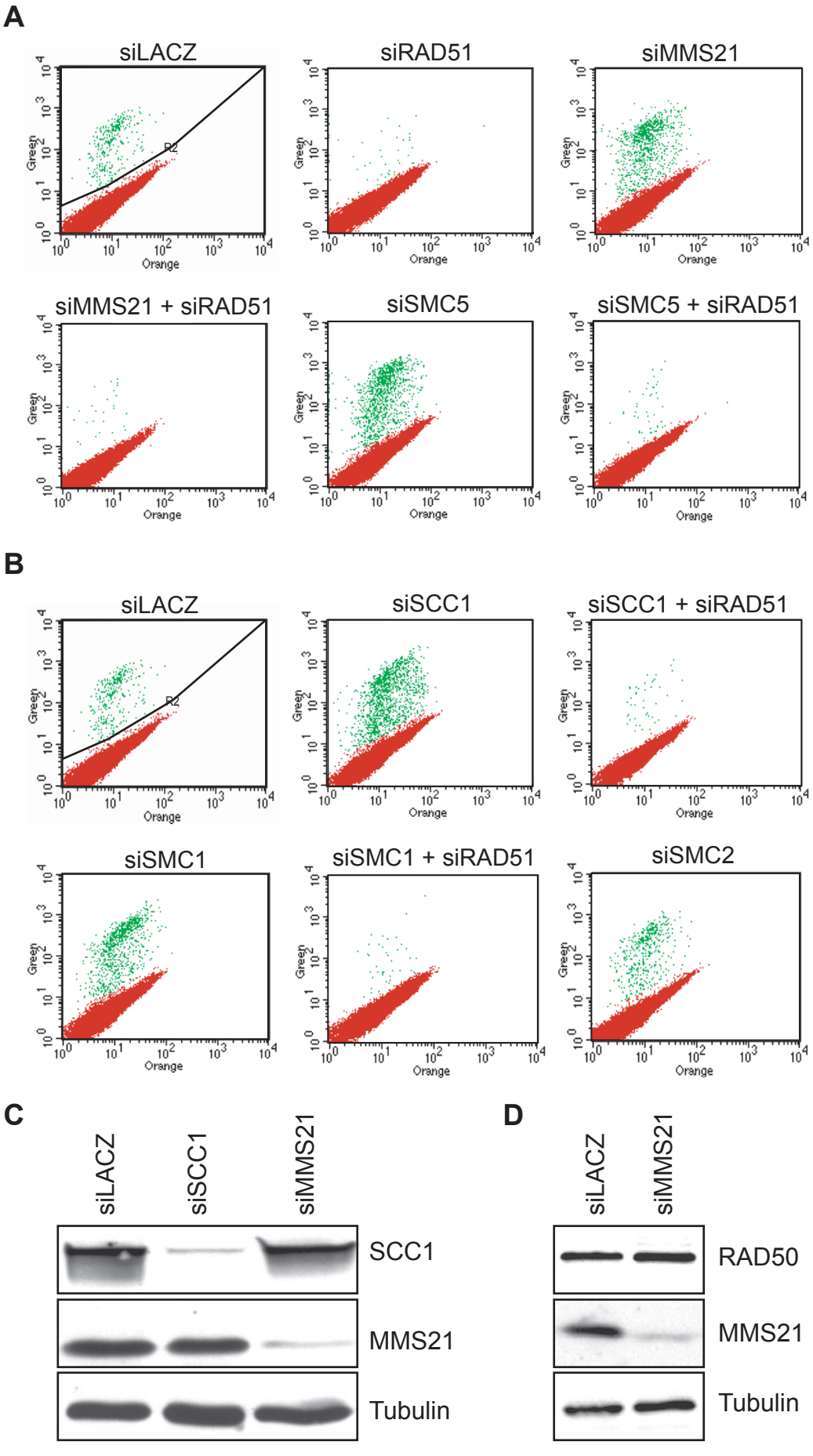
(**B**) HeLa S3 cells were transfected with mock or MMS21 siRNA for 48 hrs. Cell cycle profile of each sample was analyzed by FACS. The percentages of cells in G1, S, and G2/M are shown.

(**C**) 293/A658 cells were transfected with a ponasterone-inducible I-SceI vector for 24 hrs. Cells were then incubated with varying concentrations of vinblastine or aphidicolin for 3 hrs before the addition of ponasterone for 21 hrs to induce I-SceI expression. Cells were washed after the induction period to remove ponasterone and vinblastine or aphidicolin. Cells were allowed to recover and express GFP for 2-3 days before FACS. The percentage of GFP-positive cells is shown. Results are an average from at least three separate experiments and the standard deviations are indicated.

Supplemental Figure 5 Validation of Chromatin Immunoprecipitation (ChIP) Experiments.
(**A**) The PCR products using the *GFP* primers are specific to *GFP* and the PCR reactions are in the linear range. PCR reactions with varying amounts of input genomic DNA from either 293/A658 cells with the integrated gene targeting locus containing the *GFP* gene with the Stop-I-SceI site or wild-type 293 cells were performed.
(**B**) The sonicated fragments of DNA for ChIP were separated on agarose gel and stained with ethidium bromide.

Supplemental Figure 6 hMMS21 Stimulates the Sumoylation of Cohesin Subunits, hSCC1 and hSA2.
HeLa Tet-On cells were transfected with Myc-tagged cohesin subunits separately with or without GFP-hMMS21 wild-type or C215 SUMO ligase mutant in the presence of GFP-SUMO1 wild-type or ΔGG conjugation mutant. Cell lysates were collected 24 hrs after transfection and blotted with anti-Myc.

Supplemental Table 1 Sequences of the oligonucleotides used in this study.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siMMS21
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siSMC5
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B
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siSMC1
siSMC1 + siRAD51
siSMC2
Green
Orange
R2
C
siLACZ
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RAD50
MMS21
Tubulin

Figure S1

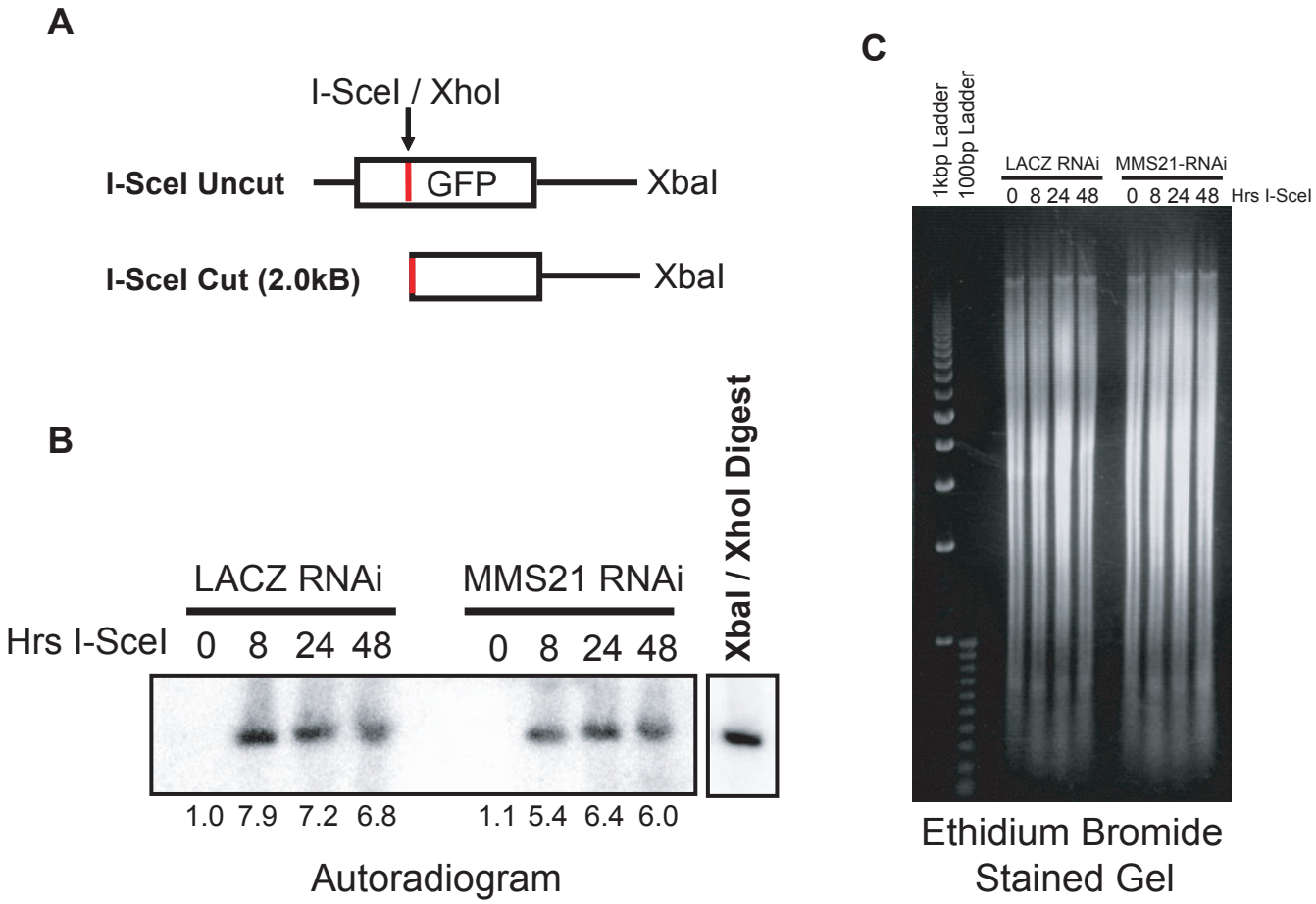
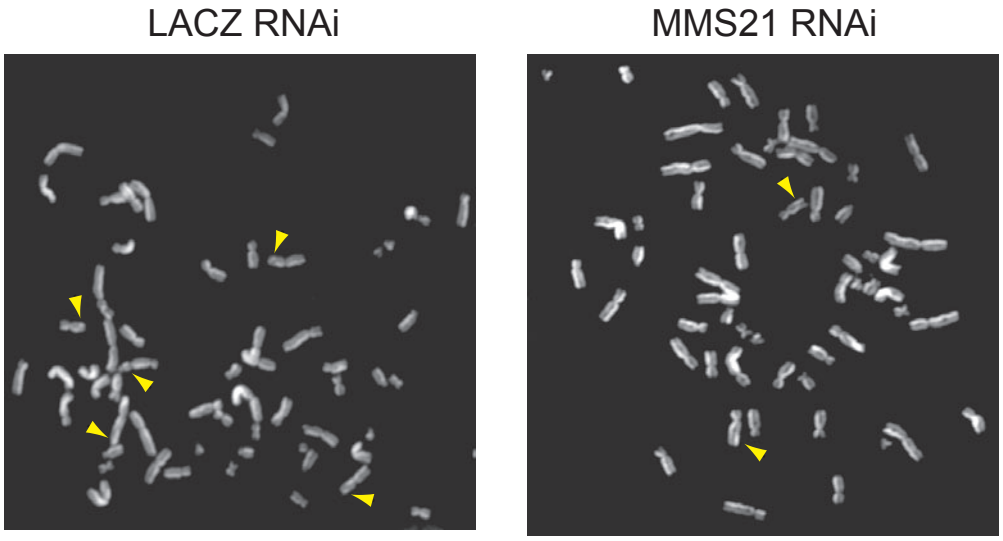

A
I-SceI / XhoI
I-SceI Uncut
GFP
XbaI
I-SceI Cut (2.0kB)
XbaI
B
XbaI / XhoI Digest
LACZ RNAi
MMS21 RNAi
Hrs I-SceI
0 8 24 48
0 8 24 48
1.0 7.9 7.2 6.8
1.1 5.4 6.4 6.0
Autoradiogram
C
1kbp Ladder
100bp Ladder
LACZ RNAi
MMS21-RNAi
0 8 24 48
0 8 24 48
Hrs I-SceI
Ethidium Bromide
Stained Gel

Figure S2

A



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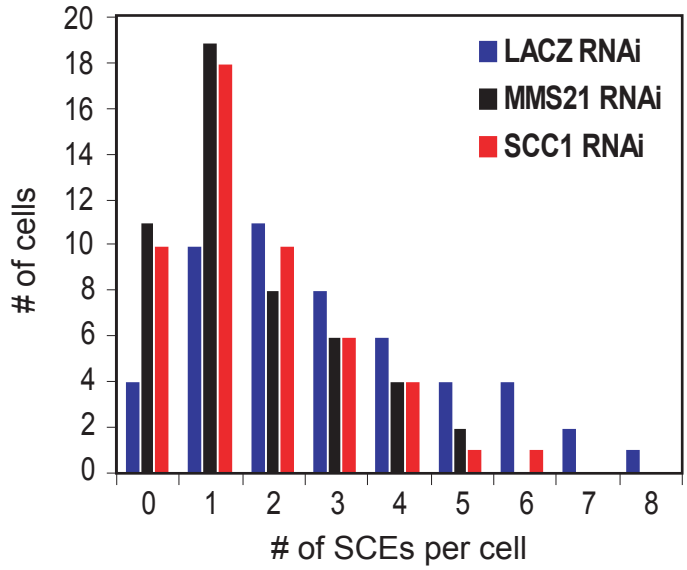


RNAi	# SCEs per Cell
LACZ	2.92 ± 1.56
MMS21	1.56 ± 1.36
SCC1	1.63 ± 1.40

C

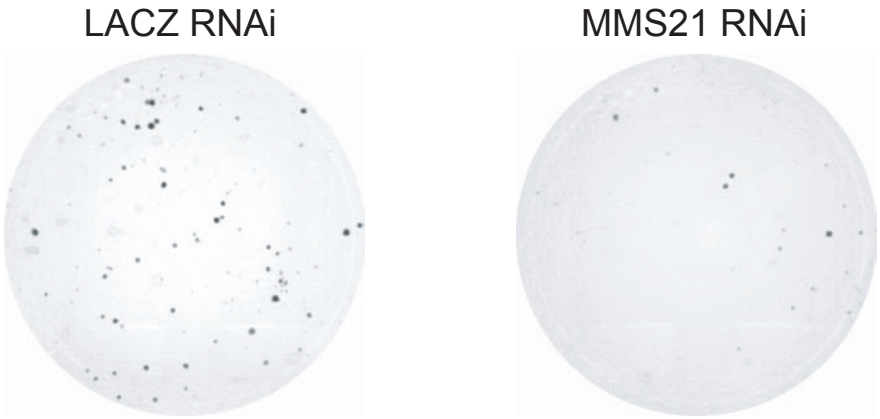


Figure S3

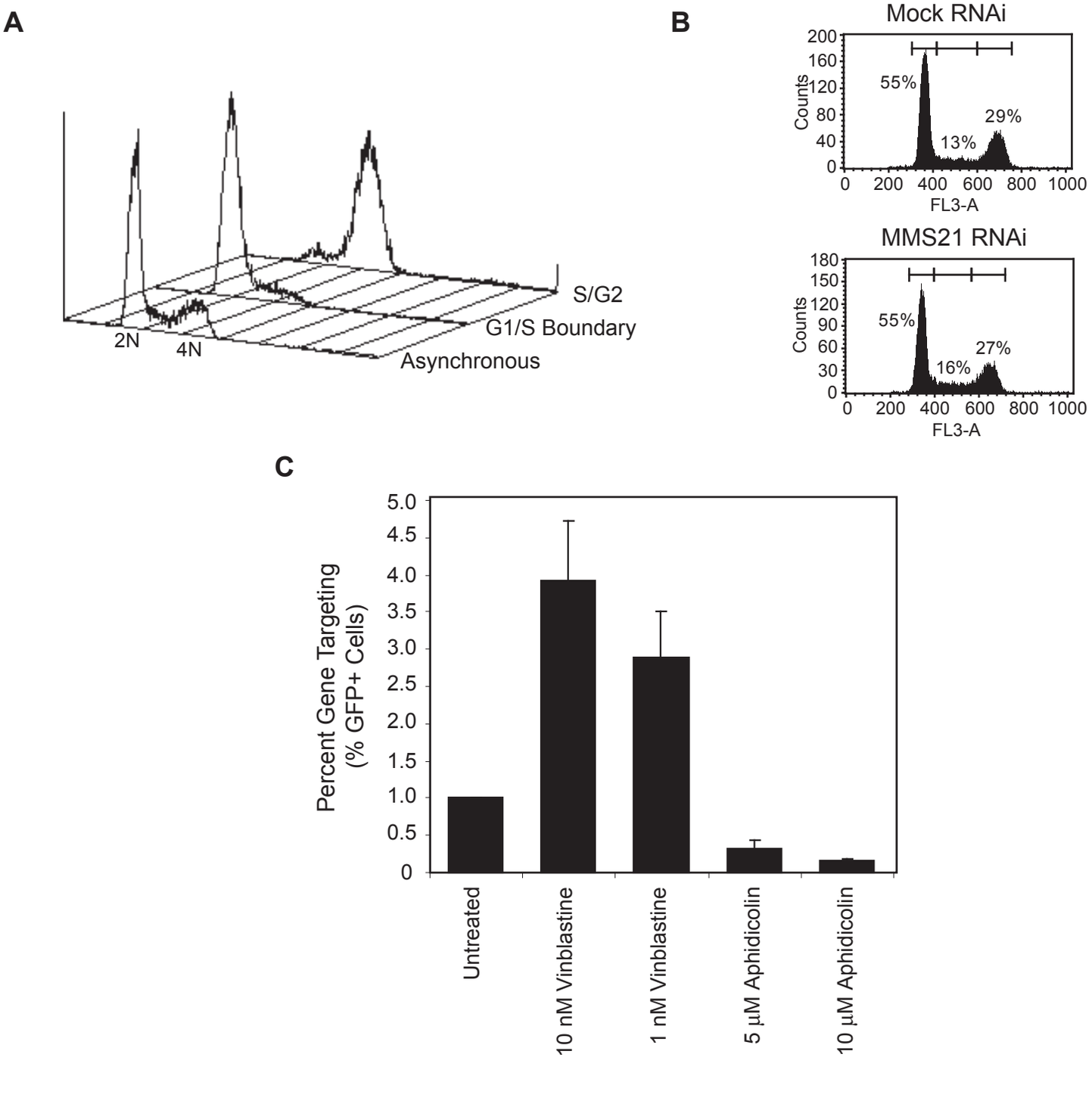
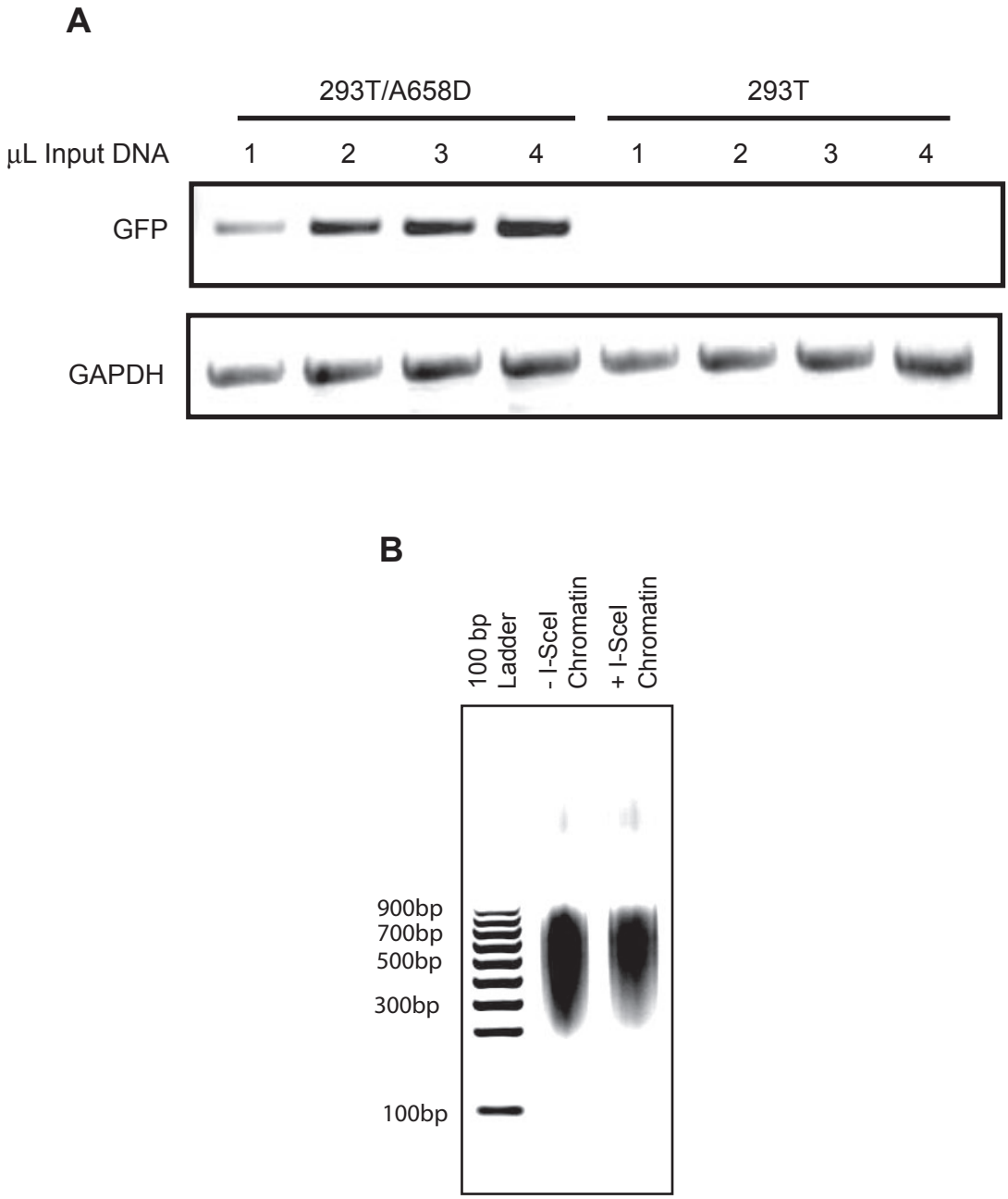

A
2N
4N
S/G2
G1/S Boundary
Asynchronous
B
Mock RNAi
Counts
55%
29%
13%
FL3-A
MMS21 RNAi
Counts
55%
27%
16%
FL3-A
C
Percent Gene Targeting
(% GFP+ Cells)
Untreated
10 nM Vinblastine
1 nM Vinblastine
5 μM Aphidicolin
10 μM Aphidicolin

Figure S4


A
293T/A658D
293T
µL Input DNA
1
2
3
4
1
2
3
4
GFP
GAPDH
B
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- I-SceI Chromatin
+ I-SceI Chromatin
900bp
700bp
500bp
300bp
100bp

Figure S5

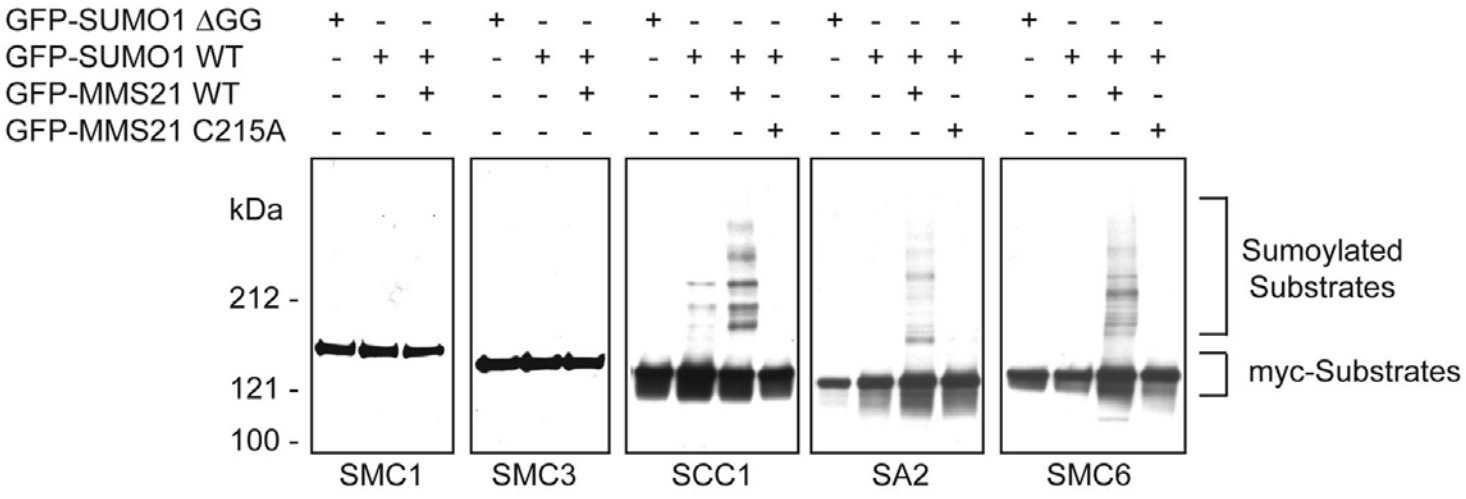
GFP-SUMO1 ΔGG
GFP-SUMO1 WT
GFP-MMS21 WT
GFP-MMS21 C215A
kDa
212 -
121 -
100 -
SMC1
SMC3
SCC1
SA2
SMC6
Sumoylated Substrates
myc-Substrates

Figure S6

Primers for Conventional PCR and QPCR	
GFP #1 F	5’-CGT CCA GGA GCG CAC CAT CTT CTT-3’
GFP #1 R	5’-ATC GCG CTT CTC GTT GGG GTC TTT-3’
GFP #5 F	5’-GTG ACC ACC TTC ACC TAC GG-3’
GFP #5 R	5’-AAG TCG TGC TGC TTC ATG TG-3'
GFP #7 F	5’-ACA TGG TCC TGC TGG AGT TC-3’
GFP #7 R	5’-CTT GTA CAG CTC GTC CAT GC -3’
GAPDH F	5’-GAC CCC TTC ATT GAC CTC AAC TAC A-3’
GAPDH R	5’-GGT CTT ACT CCT TGG AGG CCA TGT-3’
AluSx F	5'-AAA TGT GCT TTG AAC AAA GGA GTC-3'
AluSx R	5'-AGT TTC AGG TCT CTT GGA GAG TG-3'

Primers for LM-PCR	
BW-1	5’-GCG GTG ACC CGG GAG ATC TGA ATT C-3’
BW-2	5’-GAA TTC AGA TC-3’
Linker-2	5’-ACC CGG GAG ATC TGA ATT C-3'
GFP #3 R	5’-AGT TCA CCT TGA TGC CGT TC-3’

Oligonucleotides for RNA interference	
hMMS21	5’-CUC UGG UAU GGA CAC AGC UTT-3’
hSMC5	5’-GAA GCA AGA UGU UAU AGA ATT-3’
hSMC1	5’-CAA GCG GCG UAU UGA UGA ATT-3’
hSMC2	5’-AAA CGU CGA UAC ACU AUA ATT-3’
hSCC1	5’-GCC CAU GUG UUC GAG UGU ATT-3’
LACZ	Dharmacon SMARTpool Service
hRAD51	Dharmacon SMARTpool Service
hKU70	Dharmacon SMARTpool Service

Table S1