Supplementary material:

Supplementary Figure Legends:

Supplementary Figure S1: Validation of the specificity of anti-CHFR antibodies. (**A**) IHC analysis of HeLa (absence of CHFR expression), and HeLa cells that ectopically express HA-tagged CHFR. (**B**) Western blot analysis with cell lysates from T47D cells transduced with either CHFR retroviral shRNA or control vector using indicated antibodies.

Supplementary Figure S2: Oligonucleotide primers used for amplification and sequencing of *Chfr* in mouse tumors.

Supplementary Figure S3: Chromosomal aberrations of MEFs with *Chfr/Mlh1* deficiencies. (**A**) Spontaneous chromosomal aberrations were evaluated in MEFs with indicated genotypes. Two different MEF lines were used for each genotype (filled bars, MEF line1; open bars, MEF line2). Number of chromosomal aberrations per chromosome is presented. (**B**) DNA-damage-induced chromosomal aberrations were scored in MEFs with indicated genotypes. MEFs were treated with a low dose of ionizing radiation (IR) (1 Gy) and allowed to recover for 1 hour. Two different MEF lines were used for each genotype (filled bars, MEF line1; open bars, MEF line2). Number of chromosomal aberrations per chromosome is presented.

Supplementary Figure S4: Deficiency in *Chfr* sensitizes cells to antimicrotubule drug treatment. MEFs ($Chfr^{+/+}Mlh1^{+/+}$, $Chfr^{-/-}Mlh1^{+/+}$, $Chfr^{+/+}Mlh1^{-/-}$, and $Chfr^{-/-}Mlh1^{-/-}$) were treated with diluent (DMSO) or increasing concentration of either nocodazole (**A** and **C**) or MNNG (**B** and **D**). (**A** and **B**) Cell viabilities were assessed after 72h by MTS assay as described in "Methods". (**C** and **D**) Apoptotic response to the treatments was determined by staining with FITC-Annexin V and PI binding, followed by flow cytometry. Two different MEF lines per genotype were analyzed for each concentration. Experiments were performed in triplicate. Each data point was calculated from three triplicate samples per treatment group for each MEF line. *Bars*, SD of triplicate samples. Representative experiment out of three is shown.

Figure S1

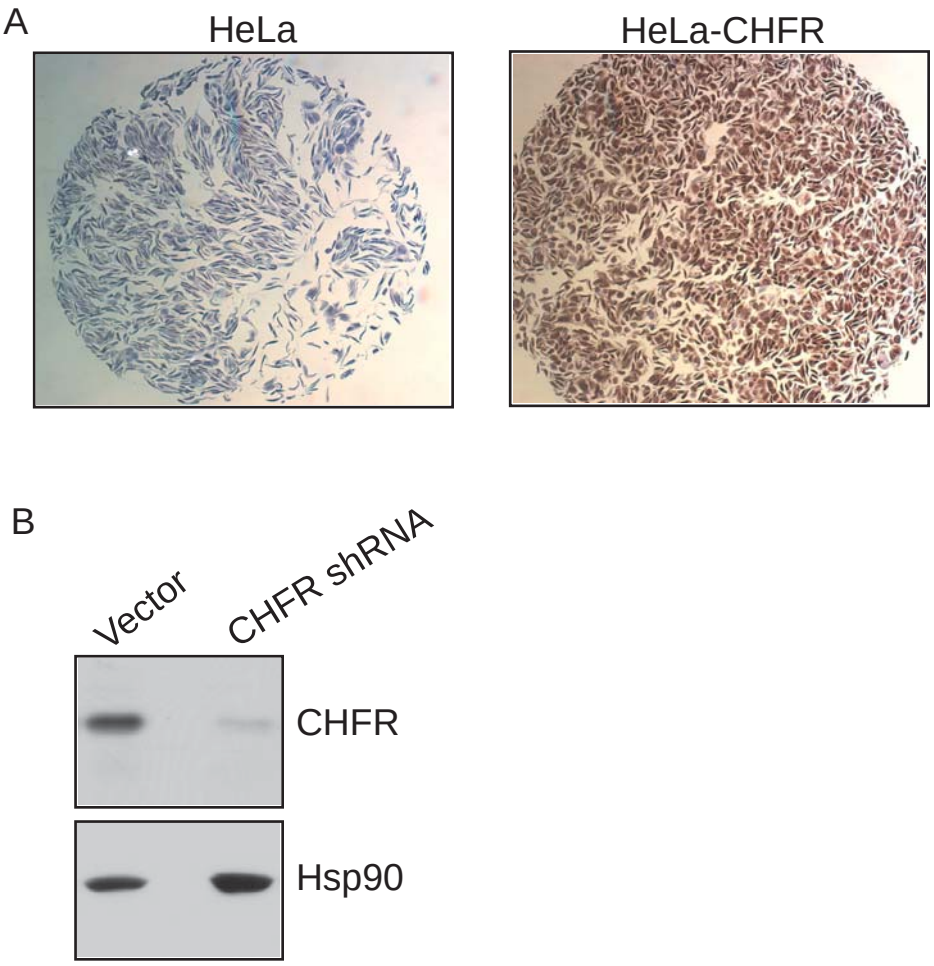


Figure S2

Promoter/Exon Covered	Nucleotide sequence		Product size (bp)
	Forward	Reverse	
promoter_1	CGCTGTCAACAGACATCTCG	GGCATTGTTTTTGCCAGTGT	595
promoter_2	GCCCAGCCAATCACATTAGA	TCGTCTCTGAGGGCTGATTT	598
Exon1-2	CCAATCACAGGGCGTACC	CCCTGTCTACCCCTTTAGCC	447
Exon3	TTGGTCACCGACTGGAAAAG	GCTGAGAAAACCATAAGCAGG	293
Exon4	CTGGGCAAATTTCTTAATATGG	TCTTGGTGCAACACCTGG	270
Exon5	GAGTTGACTTCGCACTTCCC	CCATCCTAGAACACAAAGCCC	178
Exon6	GAGTTGACTTCGCACTTCCC	CAGAACACTCACTGATGAATGG	534
Exon7	CATGGATGAGTAGATAAGACGTGC	AACCTAGTGCAGGCAACAGC	378
Exon8	GAGAAATGTGAGGTCGTCGG	ACACTTGTCAAGGGAGCCAG	289
Exon9	TCACAGTGGCAGATCTTTGG	GATGATGGCTCAGGGCATAG	286
Exon10	AAAGGCAAAACCTCAACACTG	ACCCCATAGCACAGTGAAGC	302
Exon11	TGATATCTGCATGTCTGCCTG	GGAAAATAGAACAACAATCACAAGG	286
Exon12	TTGTCTGCTGTCTTTCACCTG	CCACTCAGCTACGTCTCTATCC	285
Exon13	GCTCTTGAGTCTGGTGCTAAATG	ACACAATCCAACACCACCG	254
Exon14	GGCAAACAAGTTTGATAAGATGC	GAAACCACACAAGTTATAGGAAAGC	209
Exon15	TCCCTGACTAGTTGAGTGGTTC	CACTTCATAGGAAACTAAGACACCC	227
Exon16	GGTGTTAGAAAATGACATTGCTTG	CCATCCCAAGTGCCTGTTAG	240
Exon17	TCCTTTAGCTACATGAAAGGAATATG	TTACTCTTGTCTTCACAAGCCC	208
Exon18_1	CATGGGGCAATTACAGTGTG	CCTATGAGCTTCCCATTTTAACC	488
Exon18_2	CTCCCTCCTGTGTGCTGAG	GTTTCAGCAGGGAGTTGCTC	489
Exon18_3	TGGAAGAATGAGCATATGTTTCTG	TGAAGACTCAGTGGTGCCTG	531

Figure S3

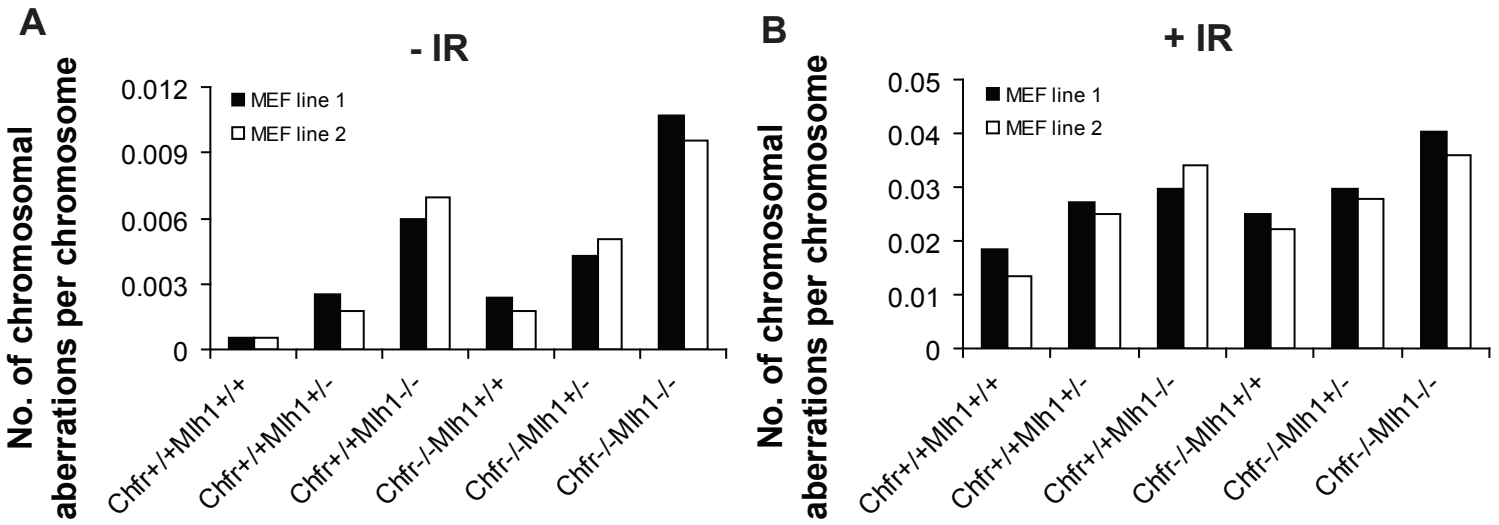
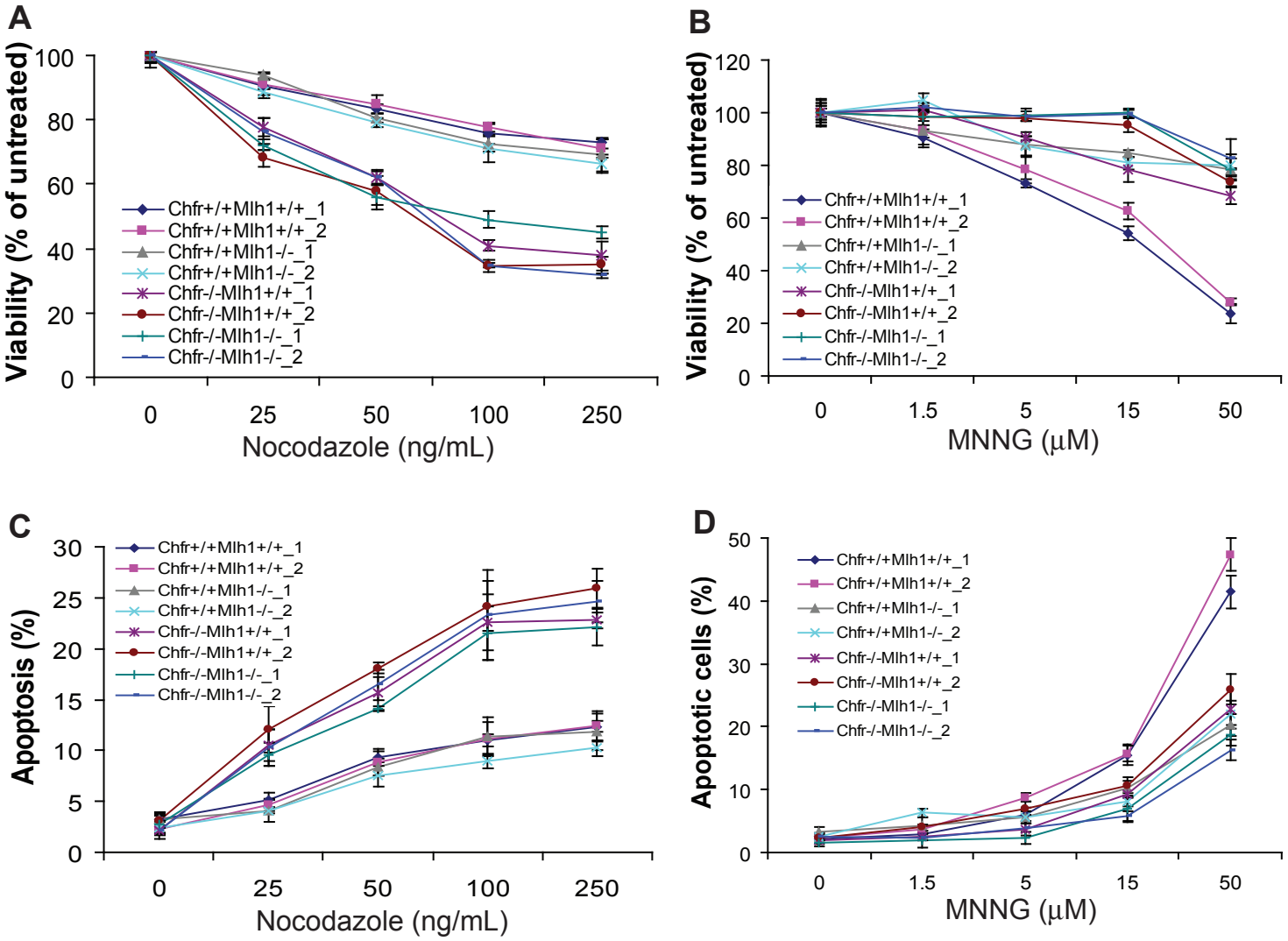

A
- IR
No. of chromosomal aberrations per chromosome
0.012
0.009
0.006
0.003
0
MEF line 1
MEF line 2
Chfr+/+Mlh1+/+
Chfr+/+Mlh1+/-
Chfr+/+Mlh1-/-
Chfr-/-Mlh1+/+
Chfr-/-Mlh1+/-
Chfr-/-Mlh1-/-
B
+ IR
No. of chromosomal aberrations per chromosome
0.05
0.04
0.03
0.02
0.01
0
MEF line 1
MEF line 2
Chfr+/+Mlh1+/+
Chfr+/+Mlh1+/-
Chfr+/+Mlh1-/-
Chfr-/-Mlh1+/+
Chfr-/-Mlh1+/-
Chfr-/-Mlh1-/-

Figure S4


A
Viability (% of untreated)
100
80
60
40
20
0
0
25
50
100
250
Nocodazole (ng/mL)
Chfr+/+Mlh1+/+_1
Chfr+/+Mlh1+/+_2
Chfr+/+Mlh1-/-_1
Chfr+/+Mlh1-/-_2
Chfr-/-Mlh1+/+_1
Chfr-/-Mlh1+/+_2
Chfr-/-Mlh1-/-_1
Chfr-/-Mlh1-/-_2
B
Viability (% of untreated)
120
100
80
60
40
20
0
0
1.5
5
15
50
MNNG (μM)
C
Apoptosis (%)
30
25
20
15
10
5
0
0
25
50
100
250
Nocodazole (ng/mL)
D
Apoptotic cells (%)
50
40
30
20
10
0
0
1.5
5
15
50
MNNG (μM)