Supporting Information

Kim et al. 10.1073/pnas.1114483108

SI Materials and Methods

Construction of Transformation-Associated Recombination Cloning Vectors. The transformation-associated recombination (TAR) circularizing vectors, pVC-NBS1 and pVC-VHL, which contain 5′ and 3′ sequences of the human *NBS1* and *VHL* genes, were constructed using the basic vector pVC604. The 307-bp ApaI-XmaI and 193-bp XmaI-XbaI fragments corresponding to 5′ and 3′ regions of *NBS1* were inserted into the polylinker of pVC604. ATG and stop codons are located approximately 5 kb upstream of the 5′ hook and approximately 1.5 kb downstream of the 3′ hook of *NBS1*. The 5′ and 3′ targeting sequences of *NBS1* were designed based on the available information (March 2006, NCBI36/hg18) and correspond to positions 91,070,470 to 91,070,776 and 91,015,482 to 91,015,674 on the chromosome 8 sequence. Size of the targeted genomic fragment containing the *NBS1* gene is 55,294 bp. The 214-bp XhoI-SmaI and 126-bp SmaI-XbaI fragments corresponding to 5′ and 3′ regions of *VHL* were inserted into the polylinker of pVC604. The 5′ and 3′ targeting sequences of *VHL* were developed based on the available information (March 2006, NCBI36/hg18) and correspond to positions 10,148,615 to 10,148,828 and 10,173,838 to 10,173,963 on chromosome 3. ATG and stop codons are located approximately 9.9 kb upstream of the 5′ hook and approximately 7.3 kb downstream of the 3′ hook of VHL. Size of the targeted genomic fragment containing the *VHL* gene is 25,348 bp. The targeting sequences were cloned into vector pVC604 in orientations corresponding to their orientations in the genome. The TAR vectors were linearized with XmaI or SmaI (the site is located between the targeting sequences) before transformation to yield a molecule bounded by the gene sequences.

Yeast Strain and Transformation. For transformations, the highly transformable *Saccharomyces cerevisiae* strain VL6–48 (MATα, his3-Δ200, trp1-Δ1, ura3-52, lys2, ade2-101, met14) that has *HIS3* deleted was used. For cloning of genomic copies of the *NBS1* and *VHL* genes, aliquots (~60 μL) containing approximately 2 to 3 μg of high molecular weight human DNA were prepared, mixed with a linearized TAR vector (1 μg), and presented to freshly prepared yeast spheroplasts. Yeast transformants were selected on synthetic complete medium plates lacking histidine. Ten transformation experiments were carried out, and approximately 1,000 colonies were obtained. (The yield of transformants per 2–3 μg of human DNA using 1 μg of vector and 5×10^8 spheroplasts varied between 10 and 50.) To identify gene-containing clones, the transformants were combined into pools and examined with the diagnostic primers for the unique *NBS1* or *VHL* sequences not present in the vector by PCR (Table S1). Five to seven gene-positive pools were identified. The PCR products were sequenced and found to match the expected *NBS1* or *VHL* sequences. Individual clones containing the *NBS1* or *VHL* genes were found in each gene-positive pool and used for further analysis. The *NBS1* and *VHL* coding regions of TAR/YAC clones were examined by PCR with specific pairs of primers for gene exonic regions (Table S1).

Construction of pJBRV1 Retrofitting Vector and Conversion of NBS1- and VHL-YACs into BACs Containing a loxP Site. A diagram of the pJBRV1 vector and retrofitting of a circular YAC into a BAC is shown in Fig. S2. Vector pJBRV1 contains the 3′ end of the *HPRT* gene, an eGFP cassette, a lox P sequence, and two short targeting hooks (~300 bp each), separated by the unique BamHI site, that flank the *ColE1* origin of replication in the pVC604-based TAR cloning vector. The hooks are homologous to the vector sequences of pVC604. Recombination of the BamHI-linearized pJBRV1 vector with a YAC in yeast leads to replacement of the *ColE1* origin of replication in the TAR cloning vector by a cassette containing the F factor origin replication, the chloramphenicol acetyltransferase (Cm^R) gene, and the *URA3* yeast selectable marker. A standard lithium acetate transformation procedure was used for retrofitting of YACs into BACs. YAC retrofitting was highly efficient: more than 95% of Ura^+ His^+ transformants obtained with pJBRV1 contained retrofitted YACs. The YAC/BACs were moved to *Escherichia coli* by electroporation. In brief, yeast chromosome-size DNAs were prepared in agarose plugs and, after melting and agarase treatment, the DNAs were electroporated into DH10B competent cells (Gibco/BRL) by using a Bio-Rad Gene Pulser.

Physical Characterization of YAC and BAC Clones. Several approaches were taken to establish the integrity and stability of the cloned material in the TAR isolates. To check the size of inserts in the gene-positive clones, chromosomal-size DNAs from yeast transformants and *E. coli* cells were digested with NotI, separated by clamped homogeneous electrical field electrophoresis, and stained with EtBr in the case of BACs or blotted and hybridized with the probes specific to either the *NBS1* or *VHL* gene in the case of YACs. To identify fragments containing *Alu* sequences (*Alu* profiles), 1 μg of total yeast DNA was digested to completion with TaqI. Samples were resolved by gel electrophoresis, transferred to a nylon membrane, and hybridized with an *Alu* probe as described previously (1). To prove the presence of all exons in the *NBS1* and *VHL* genes after gene loading into the human artificial chromosome (HAC), each HPRT-positive clone was checked by PCR with exon-specific primers (Table S1). In addition, to demonstrate that the structure of the *NBS1* and *VHL* genomic segments remains intact after loading into the HAC, the *NBS1* and *VHL* gene sequences were re-TAR cloned from HACs maintained in CHO cells by using the same TAR vectors used for gene isolation from the human genome. Then, DNAs from these TAR isolates were digested by TaqI, resolved by gel electrophoresis, and hybridized with an *Alu* probe.

Loading of Genomic Fragments into Unique loxP Site of Alphoid Tetracycline Operator HAC. A total of 3 to 5 μg of the BAC vector containing *VHL* or *NBS1* genes and 1 to2 μg of the Cre expression pCpG-iCre vector (2) were cotransformed into *hprt*-deficient CHO cells (10^5) containing the alphoid tetracycline operator (alphoid^{tetO})-HAC vector (3) by lipofection with FuGENE^{R}HD transfection reagent (Roche) or Lipofectamine 2000 (Invitrogen). *HPRT*-positive colonies were selected after 2 to 3 wk growth in HAT medium. The number of colonies obtained for *VHL* was seven, with three for *NBS1*. Insertion of the genes was confirmed by genomic PCR with a specific pair of primers that diagnose reconstitution of the *HPRT* gene (Table S1). The *HPRT* gene was reconstituted in nine of 10 analyzed clones (90%), indicating a high efficiency of accurate gene loading.

Microcell-Mediated Chromosome Transfer. alphoid^{tetO}-HAC/NBS1 and alphoid^{tetO}-HAC/VHL were transferred from CHO cells to GM07166 and renal carcinoma cell (RCC) 786-0 cell lines deficient for the *NBS1* and *VHL* genes, respectively, using a standard microcell-mediated chromosome transfer (MMCT) protocol

(4). Blasticidin (BS) was used to select resistant colonies. Typically, three to 10 BS^R colonies were obtained in one MMCT experiment involving HAC transfer into the gene-deficient cells. At least three BS^R clones were analyzed by FISH for each HAC. Based on FISH analysis, more than 90% of cells in the colony contained a HAC. In 95% of these cells, the HAC was maintained as one copy. Cotransfer of CHO chromosomes was never detected in our experiments using a sensitive PCR test for rodent *SINE* elements (Table S1).

FISH Analysis. FISH and immuno-FISH analyses were performed as previously described (5, 6). HAC-containing cells were cultured in medium with 0.1mg/mL of colcemide (no. 15212-012; Invitrogen) for 3 h at 37 °C. Metaphase cells were trypsinized and collected by centrifugation for 5 min at 483 × *g*, treated in hypotonic solution (0.075 mM KCl) for 10 min at room temperature and fixed in methanol:acetic acid (3:1) solution. Cells were diluted to the appropriate density with fixative solution, spread onto precleaned slides (12-544-7; Fisher Scientific), and allowed to dry at room temperature, followed by baking in an oven for 3 h at 60 °C. Metaphase chromosomes on the slide were denatured by denaturation buffer: 70% formamide/2× SSC for 6 min at 78 °C. Samples were dehydrated through a 70%, 90%, and 100% ethanol series for 4 min each and left to air-dry. Dig-labeled (11093088910; Roche) and biotin-labeled (11093070910; Roche) probes in hybridization solution were denatured at 70 °C for 10 min and allowed to renature at 37 °C for 30 min. The hybridization mix probe was applied to the sample and incubated at 37 °C overnight. Slides were washed with 30% formamide/2× SSC four times for 5 min at 37 °C and rinsed with 2× SSC briefly. Hybridized samples were blocked with 5% skim milk/4× SSC for 1 h and subjected to the detection solution mixture: anti–digoxigenin-rhodamine Fab fragments (11207750910; Roche) and fluorescein avidin DN (A-3101; Vector Labs) in blocking solution for 45 min. Slides were washed with 4× SSC for 10 min. The metaphase chromosomes were washed with 0.1% Triton X-100/4× SSC for 10 min and rinsed with 4× SSC. The samples were counterstained with 1 mg/mL DAPI (D1306; Invitrogen) and mounted in Vectashield mounting medium (H1000; Vector Labs). Slides were analyzed by using fluorescence microscopy.

Antibodies. The following antibodies were used for immunoblotting and immunostaining: VHL (NB100-485), HIF2-α (NB100-122), CyclinD1 (RB9041), Cdk1 (sc-53), PGK1 (H00005230-A01), CA9 (NB100-417), NBS1 (NB100-60654), ATM (ab17995), ATM-S1981 (ab81292), KAP1 (ab10484), KAP1-S824 (ab70369), p53 (ab26), p53-S15 (cs9284), γ-H2A.X (ab18311), GFP (sc8334), and β-tubulin (NB600-936).

tetO-HAC Elimination by Its Targeting with Chromatin Modifiers. Transient expression of the tTS-tet-repressor fusion protein to induce the HAC loss was performed as previously described (3, 5). After transfection with the tTS fusion construct, neomycin-resistant clones were selected in the presence of doxycycline, conditions that support proper HAC segregation and HAC stability (the tTS cannot bind to tetO sites). The HAC elimination assay was then performed by transfer of the stable transfectants to media lacking doxycycline. After 48 h of culturing in the presence of neomycin (800 μg/mL), a fraction of the HAC-less cells can be detected by loss of the GFP signal that is then confirmed by FISH. The cells that have lost the HAC were selected for the further analysis. Selection of the HAC-less clones takes approximately 2 to 3 wk. These clones were used for Western blot analysis to demonstrate that the loss of the HAC was accompanied by the restoration of the original mutant phenotypes.

RT-PCR. Transcription of the *VHL* gene from $alphoid^{tetO}$-HAC/VHL in CHO and RCC 786-0 cell lines was detected by RT-PCR by using specific primers described in Table S1.

γ-Irradiation. Exponentially growing GM07166 and GM07166 containing tet0-HAC/NBS1 cells were suspended to 5×10^5 cells/mL in complete medium. Aliquots of 10 mL were plated in 25-cm^2 flasks (Nunc) and, after 2 h, irradiated at room temperature by using a ^{60}Co γ-ray source at a dose rate of 3 Gy/min. After irradiation, flasks were placed in a 37 °C incubator and removed at various times following irradiation for analysis.

ChIP Analysis. ChIP with antibodies against CENP-A (mAN1), dimethyl histone H3 Lys4 (Upstate), trimethyl H3 Lys4 (Upstate), and trimethyl H3 Lys9 (Upstate) was carried out according to a previously described method (6) with minor modifications. Cultured cells were cross-linked in 0.5% formaldehyde for 5 min at 22 °C. After addition of 0.2 vol. of 2.5 M glycine and incubation for 5 min, fixed cells were washed with TBS buffer (25 mM Tris-Cl, 137 mM NaCl, 2 mM KCl, pH 7.4) twice and washed once with sonication buffer (20 mM Tris, pH 8.0, 1 mM EDTA, 1.5 μM aprotinin, 10 μM leupeptin, 1 mM DTT, and 40 μM MG132). Next, cells were frozen in liquid nitrogen and stored at −80 °C until use. Soluble chromatin was prepared by sonication (Bioruptor sonicator; Cosmo Bio) to an average DNA size of 0.5 kb in sonication buffer and immnnoprecipitated in IP buffer (20 mM Tris-HCl, pH 8.0, 600 mM NaCl, 1mM EDTA, 0.05% SDS, 1.0% Triton X-100, 20% glycerol, 1.5 μM aprotinin, 10 μM leupeptin, 1 mM DTT, and 40 μM MG132). Protein G sepharose (Amersham) blocked with BSA was added, and the antibody–chromatin complex was recovered by centrifugation. The recovery ratio of the immunoprecipitated DNA relative to input DNA was measured by real-time PCR using a CFX96 real-time PCR detection system (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad). Primers 5SDNA-F1 and 5SDNA-R1 for 5S ribosomal DNA, 11-10R and mCbox-4 for 11-mer of chromosome 21 alphoid DNA, Sat2-F1 and Sat2-R1 for pericentromeric satellite 2 repeat (8) tet-1 and tet-3 for the $alphoid^{tetO}$ DNA, and bsr-F and bsr-R for the marker gene (bsr) of $alphoid^{tetO}$ HAC (7) were described previously (6–8). Primers NBS1-vector-F3 and NBS1-R2 for the upstream region of NBS1 gene on $alphoid^{tetO}$ HAC and NBS1-F2 and NBS1-vector-R3 for the downstream region of NBS1 gene on $alphoid^{tetO}$ HAC are in Table S1.

Cell Lines. HT1080 human fibrosarcoma cells obtained from the American Type Culture Collection were cultured in Dulbecco modified Eagle medium (Sigma) supplemented with 10% FBS (Biowest) with 4 μg/mL Blasticidin S (Funakoshi) at 37 °C in 5% CO_2. The *HPRT*-deficient CHO cells (JCRB0218) were maintained in Ham F-12 nutrient mixture (Invitrogen) plus 10% FBS with 8 μg/mL BS (Funakoshi). After introduction of a BAC construct containing a gene of interest and the eGFP gene, CHO cells retaining the tetO-eGFP-HAC were cultured with 1× HAT medium. The RCC 786-0 cell line containing a single *VHL* allele with a frameshift mutation at codon 104 (9) was obtained from Len Neckers (National Cancer Institute, Bethesda, MD). HIF1-α is not expressed in the 786-0 cell line as a result of a spontaneous mutation (10). The GM07166 cell line containing a deletion of 5 nt in exon 6 of the *NBS1* gene, resulting in a frameshift and a truncated protein, was obtained from the Coriell Institute for Medical Research.

Statistical Analysis. *P* values were calculated by using a Student *t* test. The *P* values of enrichment levels of rDNA and Sat2 with anti-CENP-A (Fig. 5*A*) are more than 0.05, which means there is no significant difference in the enrichment levels of rDNA and Sat2 in three tested cell lines.

Target	Cell line 1	Cell line 2	*P* Value
rDNA	AB2.2.18.21	NBS1 no. 3	0.456
rDNA	AB2.2.18.21	NBS1 no. 6	0.428
rDNA	NBS1 no. 3	NBS1 no. 6	0.323
Sat2	AB2.2.18.21	NBS1 no. 3	0.360
Sat2	AB2.2.18.21	NBS1 no. 6	0.276
Sat2	NBS1 no. 3	NBS1 no. 6	0.281

The *P* values of enrichment levels of 5′ and 3′ ends of NBS1 with CENP-A antibody indicate that the enrichment of CENP-A is statistically significant in both HAC-containing lines.

Cell line	Target 1	Target 2	*P* value
NBS1#3	5′ of NBS1	3′ of NBS1	0.019
NBS1#6	5′ of NBS1	3′ of NBS1	0.048

The *P* values of enrichment levels of rDNA and Sat2 with anti-H3K9me3 are more than 0.05 that means that there is no significant difference in the enrichment levels of rDNA and Sat2 in the tested three cell lines.

Target	Cell line 1	Cell line 2	*P* value
rDNA	AB2.2.18.21	NBS1#3	0.091
rDNA	AB2.2.18.21	NBS1#6	0.462
rDNA	NBS1#3	NBS1#6	0.140
Sat2	AB2.2.18.21	NBS1#3	0.230
Sat2	AB2.2.18.21	NBS1#6	0.067
Sat2	NBS1#3	NBS1#6	0.240

The *P* values of enrichment levels of 5′ and 3′ ends of NBS1 with H3K9me3 antibody indicates that the enrichment of H3K9me3 is statistically significant in both HAC-containing lines.

Cell line	Target 1	Target 2	*P* value
NBS1#3	5′ of NBS1	3′ of NBS1	0.008
NBS1#6	5′ of NBS1	3′ of NBS1	0.017

In Fig. 3*D*, the differences in foci numbers at 24 h and 48 h are statistically significant. The results were reproduced in three independent experiments. The *P* value for each time period was calculated between GM07166 and GM07166+NBS1 HAC (+) by using a Student *t* test. The *P* values for samples at 0 h, 24 h, and 48 h were less than 0.05, which means they are statistically significant.

GM07166 vs. GM07166 + NBS1 HAC (+)

Time	*P* value
0 h	0.009
1 h	0.229
2 h	0.309
3 h	0.674
6 h	0.105
24 h	0.018
48 h	0.018

The *P* values between every pairing were also calculated: GM07166

Time	1 h	2 h	3 h	6 h	24 h	48 h
0 h	0.0004	0.0060	0.0100	0.0002	0.0067	0.1100
1 h	—	0.1224	0.0785	0.1212	0.0033	0.0000 (2.35×10^{-6})
2 h	—	—	0.0794	0.0178	0.0058	0.0001
3 h	—	—	—	0.0064	0.0127	0.0001
6 h	—	—	—	—	0.0032	0.0030
24 h	—	—	—	—	—	0.0015

GM07166 + NBS1 HAC (+)

Time	1 h	2 h	3 h	6 h	24 h	48 h
0 h	0.0006	0.0001	0.0069	0.0066	0.0248	0.0827
1 h	—	0.1720	0.0095	0.2192	0.0018	0.0007
2 h	—	—	0.1017	0.0136	0.0002	0.0001 (6.46×10^{-5})
3 h	—	—	—	0.0266	0.0122	0.0075
6 h	—	—	—	—	0.0099	0.0062
24 h	—	—	—	—	—	0.0204

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Fig. S1. Physical characterization of NBS1-containing YAC and BAC clones. (*A*) *Alu*-profile characterization of YACs containing *NBS1* obtained by TAR cloning from human genomic DNA. Three gene-positive isolates were characterized and compared in terms of *Alu* profiles of restriction fragments. Total yeast DNA was isolated from yeast clones and digested to completion with TaqI. Fragments were separated by gel electrophoresis, transferred to a nylon membrane, and hybridized with an *Alu* probe. All three randomly chosen clones exhibit identical *Alu* profiles, indicating no rearrangements in *NBS1*. (*B*) Characterization of circular *NBS1* BACs. TAR/YACs were retrofitted into BACs by BRV1 vector (1) and moved to *E. coli* cells. BAC DNAs were isolated, digested by NotI for linearization, separated by clamped homogeneous electric field gel electrophoresis, and visualized by staining with ethidium bromide. The upper bands correspond to the *NBS1* sequence and the lower bands correspond to the vector part. Three BAC clones (lanes 1–3) corresponding to three independent TAR isolates were analyzed. (*C*) Restriction digestion profiles of BAC DNAs. DNA samples were digested by EcoRI (lanes 1–3) and HindIII (lanes 4–6). Identical profiles were observed for each restriction endonuclease. (*D*) Characterization of circular *NBS1* BACs. TAR/YACs were retrofitted into BACs by pJBRV1 vector and moved to *E. coli* cells. BAC DNAs were isolated from two randomly chosen clones, digested by *Bsi*W1 for linearization, separated by clamped homogeneous electric field gel electrophoresis, and visualized by staining with ethidium bromide (lanes 1 and 2). Predicted size of the *NBS1*-containing fragment was observed.

Fig. S2. Construction of the pJBRV1 vector and retrofitting of a circular YAC into a BAC. (*A*–*C*) Construction of pJBRV1. *Age*I restriction site was replaced by NotI restriction site in the pX3.1 vector (1). Then, a NotI 3′ HPRT-loxP-eGFP fragment was cloned into the pBRV1 vector (2), resulting in pJBRV1 vector. Size of the pJBRV1 vector is approximately 18 kb. The pJBRV1 vector used for retrofitting of YACs into YAC/BAC molecules contains a 3′ end of the *HPRT* gene, an eGFP cassette, a loxP sequence, and two targeting sequences, A and B (~300 bp each), separated by the unique BamHI. Targeting sequences correspond to two regions flanking the ColE1 origin of replication in the pVC604 TAR cloning vector used for gene isolation. (*D*–*E*) YAC retrofitting. Recombination between the BamHI-linearized pJBRV1 vector and a YAC containing a gene during yeast transformation leads to replacement of the ColE1 origin of replication in the TAR cloning vector by a cassette containing the F′ factor origin of replication (BAC), the chloramphenicol acetyltransferase (Cm) gene, the *URA3* yeast selectable marker, and 3′ HPRT-loxP-eGFP cassette. A standard lithium acetate transformation procedure was used for retrofitting of YACs into BACs. YAC retrofitting was highly efficient: more than 95% of $Ura^{+}His^{+}$ transformants obtained with pJBRV1 contained retrofitted YACs. The YAC/BACs were moved to *E. coli* by electroporation as described previously (2). In brief, yeast chromosome size DNA was prepared in agarose plugs and, after melting and agarose treatment, the DNA was electroporated into *Stbl4* competent cells (Gibco/BRL) by using a Bio-Rad Gene Pulser.

1. Iida Y, et al. (2010) Human artificial chromosome with a conditional centromere for gene delivery and gene expression. *DNA Res* 17:293–301.
2. Larionov V, Kouprina N, Solomon G, Barrett JC, Resnick MA (1997) Direct isolation of human BRCA2 gene by transformation-associated recombination in yeast. *Proc Natl Acad Sci USA* 94:7384–7387.

Fig. S3. A physical map of the retrofitting pJBRV1 vector with a TAR-isolated gene insert used for gene loading into the alphoid$^{\mathrm{tetO}}$-HAC.

Fig. S4. Transcriptional analysis of the *VHL* gene inserted into the alphoid$^{\mathrm{tetO}}$-HAC. (*A*) A positive control: RT-PCR of RNA purified from human HeLa cells (lanes 1–4). A negative control: RT-PCR of RNA purified from CHO cells (lanes 5–8). Lanes 9 to 12 correspond to RNA purified from CHO cells containing alphoid$^{\mathrm{tetO}}$-HAC/VHL. The primers designed from exon 1, exon 2, and exon 3 sequences are presented in Table S1. The numbers under and above bands are a predicted size of RT-PCR products. Bands in lanes 1 and 9 correspond to almost a full-length transcript of *VHL*. All amplified fragments were gel-purified and sequenced and proved the identity of products to the human *VHL* transcripts. (*B*) RT-PCR analysis of the *VHL* gene in the VHL-deficient 786-0 cell line (lanes 1 and 3) and in the same line carrying alphoid$^{\mathrm{tetO}}$-HAC/VHL (lanes 2 and 4). The 786-0 cell line contains a single *VHL* allele with a frameshift mutation at codon 104. For RT-PCR analysis, we used specific RT-full-F/RT-full-R primers (Table S1) that amplify mutant 641 bp and full-length 642-bp transcripts of *VHL* (lanes 1 and 2) and the primers RT-WT-F/RT-WT-R (Table S1) designed in such a way that they amplify a WT (lane 4) but not a mutant allele (lane 3) of *VHL* [the forward primer starts approximately from the middle of transcript, giving the product of 346 bp in size (lane 4); annealing temperature was 68 °C].

Fig. S5. Histogram illustrating HAC loss in response to tTS expression. Targeting a transcriptional silencer (tTS) into the HAC kinetochore induces HAC loss. Cells were transfected by tTS-containing vector with the *G418* selectable marker. Nontransfected cells were killed by neomycin, and the remaining population was analyzed by FISH and FACS to quantify the HAC retention after 2 wk of culture. FISH analysis includes detection of the HAC on metaphase spreads. FACS analysis was based on measuring of florescence of the GFP protein, the gene of which was included into the HAC. Addition of doxycycline, which prevents tethering certain tetracycline repressor from binding to tetO, blocked the inactivation of HAC kinetochore by tTS.

Fig. S6. Immunoanalysis of nonirradiated cells. The NBS1-deficient GM07166 cells and the same cells with alphoid^{tetO}-HAC/NBS1 and after HAC loss were double immunostained with anti–γ-H2AX and anti-h53BP1 or anti–γ-H2AX and anti-hMRE11, or anti–γ-H2AX and anti-hNBS1 antibodies.

c

	Exon 2 (rs1063045)	Exon 5 (rs1805794)	Exon 6 (5bp deletion)	Exon 10 (rs709816)	Exon 13 (rs1061302)
HAC/NBS1	A	C	No deletion	C	G
HT1080	A	C	No deletion	C	G
	G	G	No deletion	T	A
HeLa	A	C	No deletion	C	G
	G	G	No deletion	C	A

Fig. S7. FISH analysis of alphoid^{tetO}-HAC/NBS1 transferred into HT1080 (*A*) and HeLa (*B*) cells using specific probes for BAC vector (red) and gene sequences (green). DNA was stained by DAPI (blue). (*C*) Polymorphic variants of the *NBS1* gene identified by DNA sequencing.

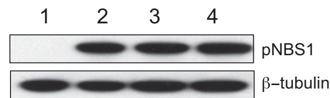

Fig. S8. Expression of pNBS1 from the HAC in NBS1-deficient cells. Western blot analysis of NBS1-deficient GM07166 cells containing a common deletion of 5 nt in exon 6 of the *NBS1* gene and the same cells carrying alphoid$^{\text{tetO}}$-HAC/NBS1 after 1, 2, and 3 mo of culturing. β-Tubulin was used as an internal control. Lane 1, GM07166 cell line; lane 2, GM07166 with alphoid$^{\text{tetO}}$-HAC/NBS1 after 1 mo; lane 3, GM07166 with alphoid$^{\text{tetO}}$-HAC/NBS1 after 2 mo; and lane 4, GM07166 with alphoid$^{\text{tetO}}$-HAC/NBS1 after 3 mo.

Table S1. Primers used in this study

Primers for pVC-NBS1 vector construction
5′ hook NBS-F1/NBS-R1 5′ CTAGCCTAGAACCTGTCATG 3′/5′ GGTCTGCTTATTGCTCCATG 3′
3′ hook NBS-F2/NBS-R2 5′ TGACATTGGATAGAACATGGG 3′/5′ GATTGTGGGCATGACAGAGA 3′
Diagnostic primers
E1-F/E1-R 5′ TCATCCAAGGCAGCCTGCGT 3′/5′TGCCATACAGCGTACTCGCC 3′
E2-F/E2-R 5′ CGTGTACATGTGTATGTGTC 3′/5′ GATTTCAACCCCCTTACTGG 3′
E3-F/E3-R 5′ CTCTGAGAAGTGAATGTACTG 3′/5′ TGGCTGAAACAAAGCTGTCC 3′
E4-F/E4-R 5′ CTGCAACTCTGATACTATGAC 3′/5′ TCTGTGTATAGTGGGTAAGC 3′
E5-F/E5-R 5′ ATGTAAACAGCCTCTTTGTAG 3′/5′ CTATCATATAAGTGACATCTT 3′
E6-F/E6-R 5′ GTGTCAGATAGTCACTCCG 3′/5′ CGTTAACAACTACTGATAAGAG 3′
E7-F/E7-R 5′ CCAAATCAAATTCTTATGTGTTC 3′/5′ CATAAAATCTCCTACTTGCAG 3′
E8-F/ E8-R 5′ TGCTTTATCTTGACATTATCTG 3′/5′ CTAGCAAGTATATGAGTAACG 3′
E9-F/E9-R 5′ GATGTTCATCTTCTATCTAGC 3′/5′ GATAAAGGGCATTACTTCCTG 3′
E10-F/E10-R 5′ CTATTAAAGTTGCTGTAAACTTG 3′/5′ TTAGTTGGTGAAAGCTGATAG 3′
E11-F/E11-R 5′ CTAAATGGTTACTTAGCTGTG 3′/5′ TTCTATGGCCACATCATCCA 3′
E12-F/E12-R 5′ CAAAAGGCCAAGAAGTGATAG 3′/5′ ATTGATGAGATGACAGTCCC 3′
E13-F/E13-R 5′ GATTCCCAAATGACAAGTGAC 3′/5′ GTTTAGCATCACTGGTATCTC 3′
E14-F/E14-R 5′ GTTAATATGATTTACTGTACTAC 3′/5′ ACCATAATGGACCAAAGTGC 3′
E15-F/E15-R 5′ ACTATATATTCACCACTGAGC 3′/5′ GAAGGGACTAGGTGTCTATG 3′
E16-F/ E16-R 5′ TACAGTTTGAAAGTTCTTTACC 3′/5′ AGATGCAATGACAAAGCCTG 3′
Exon polymorphism checking primers
Exon 2F-Exon 13R 5′ GGCAGGAGGAGAACCATACA 3′/5′ TCCTCCAATGATGTGTGGAA 3′
Seq exon 2F1 5′ GGCAGGAGGAGAACCATACA 3′
Seq exon 2F2 5′ GTCTAGCAGCCCCGGTTAC 3′
Seq exon 5F 5′ TTGGTTGCATGCTCTTCTTG 3′
Seq exon 10F 5′ CACAAGGCGTGTCAGTTGAT 3′
Seq exon 13R 5′ TCCTCCAATGATGTGTGGAA 3′
Seq exon 14R2 5′ TCCTGCCTTAGCCACTCTTC 3′
Primers for pVC-VHL vector construction
5′ hook VHL-F1/VHL-R1 5′ CCCAGGTCAGTGCAGAGCCAC 3′/5′ CGTAGCAAGCTCAAAGGAGCCA 3′
3′ hook VHL-F2/VHL-R2 5′ TGGATTACCCTGGCGTGGAGCA3′/5′GGGGGTGGAAATCCTGGAGGC 3′
Diagnostic primers for RT-PCR
dia1-F/dia1-R 5′ TGCTTCAGTGTGGCTTTCACAAACA 3′/5′TGCAGTGAAGTTGATACCACATTGCT 3′
diag2-F/diag2-R 5′ ACACCTAAACTGCCTGCCCCA 3′/5′ CTCCAGGCCCCCAGGATTCA 3′
E1-F/E1-R 5′ GCCCTGAAGAAGACGGCGGG 3′/5′ CAGGCGGCAGCGTTGGGTAG 3′
E2-F/E2-R 5′ GTCACCTTTGGCTCTTCAGAGATGC 3′/5′ CTGGCAGTGTGATATTGGCAAAAATAG 3′
E3-F/E3-R 5′ TCAATCTCCCATCCGTTGATGTG 3′/5′ TGTATACTCTGAAAGAGCGATGCCTC 3′
RT full F/RT full R 5′ ATGCCCCGGAGGGCGGAGAAC 3′/5′ TCAATCTCCCATCCGTTGATGTG 3′
RT WT F/RT WT R 5′ AACGCTGCCGCCTGGCACG 3′/5′ TCAATCTCCCATCCGTTGATGTG 3′
VHLex1F/VHLex3f 5′ GCCCTGAAGAAGACGGCGGG 3′/ 5′ TCAATCTCCCATCCGTTGATGTG 3′
VHLex1F/VHLex1R 5′ GCCCTGAAGAAGACGGCGGG 3′/5′CAGGCGGCAGCGTTGGGTAG 3′
VHLex2rev/VHLex3f 5′ GTCACCTTTGGCTCTTCAGAGATGC 3′/ 5′ TCAATCTCCCATCCGTTGATGTG 3′
VHLstart/ VHLex1R 5′ ATGCCCCGGAGGGCGGAGAAC 3′/ 5′CAGGCGGCAGCGTTGGGTAG 3′
Primers for NotI site insertion
NotI F 5′ AGATCACCGGTAACGGAGCTGAATGAAGCATGCGGCCGCGATGGGTCTGGAGCGTGAGCACCGGTATGCA 3′
NotI R 5′ TGCATACCGGTGCTCACGCTCCAGACCCATCGCGGCCGCATGCTTCATTCAGCTCCGTTACCGGTGATCT 3′
Primers for CHO DNA contamination after MMCT
Cons B2 5′ CCATCTGTAATGAGATCTGATGC 3′
Ham B2-F/Ham B2-R 5′ GCTCAGAGGTTAAGAGCACTGAC 3′/5′ TGCTTCCATGTATATCTGCACAC 3′
Primers for HPRT reconstitution
Lox137-R/Rev no 6 5′AGCCTTCTGTACACATTTCTTCTC 3′/ 5′GCTCTACTAAGCAGATGGCCACAGAACTAG 3′
Lox137-R/SV40 PA 5′AGCCTTCTGTACACATTTCTTCTC 3′/5′AATGGTTACAAATAAAGCAATAGCATCAC 3′
Primers for ChIP analysis of 5′ and 3′ ends of the NBS1 gene in tetO-HAC
NBS1-vector-F3/NBS1-R2 5′ AGCGGATAACAATTTCACACAGGA 3′/5′ GGGTCTTGACAGAGTCTTCCC 3′
NBS1-F2/NBS1-vector-R3 5′ TCATAATTACCAAGTGAAGCTGGTGG 3′/5′CACGACGTTGTAAAACGACGGC 3′