SUPPLEMENTAL INFORMATION

Supplemental methods

Exome sequencing and sequence data analysis

Sequence capture, enrichment and elution was performed using the Agilent in-solution enrichment methodology (SureSelect Human All Exon Kits Version 5-UTRs, Agilent) according to manufacturer's instructions without modification except for library preparation performed with NEBNext Ultra kit (New England Biolabs). For library preparation, 600 ng of genomic DNA was fragmented by sonication and purified to yield fragments of 150-200 bp. Paired-end adaptor oligonucleotides from the NEB kit were ligated on repaired, A tailed fragments then purified and enriched by 8 PCR cycles. 1,200 ng of these purified libraries were then hybridized to the SureSelectoligo probe capture library for 72 hrs. After hybridization, washing, and elution, the eluted fraction was PCR-amplified with 9 cycles, purified and quantified by qPCR. Appropriate amount of eluted-enriched DNA was sequenced on an IlluminaHiSEQ 2000 as paired-end 75 bases reads. Image analysis and base calling was performed using Illumina Real Time Analysis (RTA 2.1.3) with default parameters.

The bioinformatics analysis of sequencing data was based on the Illumina pipeline (CASAVA1.8). CASAVA performs alignment of the reads to the human reference genome (hg19) with the alignment algorithm ELANDv2 (performs multiseed and gapped alignments), then calls the SNPs based on the allele calls and read depth, and detects variants (SNPs and Indels). Only the positions included in the bait coordinates were conserved. Variant annotations were performed using IntegraGen in-house pipeline, which consists on gene annotation (RefSeq), detection of known polymorphisms (dbSNP 132, 1000Genome) followed by a mutation characterization (exonic, intronic, silent, nonsense). Copy number abnormality analysis was also performed to search for deletion and duplications.

PCR, RNA extraction and qRT-PCR

RNA samples were reverse-transcribed (Superscript vilo; Invitrogen) and the cDNA was analyzed for expression by quantitative PCR (qRT-PCR) using SYBR green ready mix (Applied Biosystems). Gene expression values were normalized to the expression of HPRT used as reference gene. Levels were indicated relative to HPRT expression value using the ΔCT method. All primers are listed in Table S1.

Constructs

A *REV7* cDNA was amplified by PCR and cloned downstream the EF1α promoter into an HIV-derived lentiviral vector (pRRLsinEF1a-PGK-eGFP-WPRE; Genethon, France) as previously described (33). Gene silencing in murine hematopoietic cells was performed using shRNAs targeting murine *Rev7* (13) cloned in lentiviral vector as previously described (33). Cells were cultured in RPMI supplemented with 20% BIT (Stem Cell Technologies), stem cell factor, Flt3-ligand, IL-3 (R&D Systems Europe), and thrombopoietin (Stem Cell Technologies). Silencing efficiency was checked by qRT-PCR.

Functional in vitro tests in EBV cells

Analyses were performed, without or after 1hr MMC pulse (100 ng/mL) on growing cells, in at least two independent experiments. For cell-cycle analysis, cells were treated with MMC, washed and cultured in MMC-free medium. At the indicated time-points, cells were isolated and re-suspended in lysis buffer (Triton 0.1%, 1 mg/mL of sodium citrate) containing RNAse (20 mg/mL) and PI (20 mg/mL). Ratios of cycling cell were analyzed by flow cytometry. Chromosomal breaks tests were scored on mitoses using conventional cytogenetic methods (34,35). EBV cell lines and fibroblasts were transduced with a sinPRRLlentivirus encoding CHERRY. $Cherry^+$ cells were sorted and cultured.

Immunoblotting and immunofluorescence

Immunoblotting. Antibodies were used against human p^{Ser15}p53 (Cell Signaling), Actin (Santa Cruz), human FANCD2 (Novus Biologicals), γH2AX (Millipore), 53BP1 (Cell signaling), REV7 (abcam). Signals were detected using an enhanced chemiluminescence (ECL) kit (Pierce) and visualized with a Vilber Lourmat luminescent image analyzer system. *Immunofluorescence*. Fibroblasts (30,000 cells per well) were seeded in eight-well chamber slides (Labtek). After overnight culture, cells were cultured or not with MMC (50ng/mL), 48hrs later, cells were washed with PBS1X. Cells were fixed with 4% paraformaldehyde (15 min at room temperature) and permeabilized with 0.5% Triton X-100 in PBS (10–20 min at room temperature). Unspecific binding sites were blocked with PBS1X + 5% BSA for 1 hr at room temperature. Slides were incubated with specific antibodies in blocking buffer (2 hrs at room temperature). Slides were then incubated with secondary antibody labeled with Alexa 488 (Invitrogen, 1:1,000) for 1 hr at room temperature. Finally, slides were washed four times with PBS1X and embedded. Slides were analyzed with a fluorescent microscope Axiovert 2000M (Carl Zeiss MicroImaging).

Sequence analysis

For direct sequencing, PCR products were generated by *REV7* exon5 specific primers (Supplemental Table S1).

Functional tests on REV7$^{-/-}$ U2OS cells

Chromosomal radials and aberrations were evaluated using conventional cytogenetics on metaphase spreads following 48 hours treatment with 20 ng/mL MMC. For cell cycle analysis, cells were either untreated or treated with 20 ng/mL MMC for 48 hours. Cells were then fixed in 70% EtOH overnight at 4°C. Fixed cells were rinsed and resuspended in FxCycle PI/RNase Staining Solution (Life tech Cat #F10797) and analyzed using a BD FACScalibur. Cell cycle data was processed using FlowJo software. For clonogenic survival assays, 1000 cells were plated per well in 6-well plates. The next day, the cells were treated

with MMC and grown in the presence of MMC for 10 days. Cells were then fixed with crystal violet, and colonies were counted.

In vitro differentiation assays

Fancg$^{-/-}$ were a kind gift from Freerk Arwert, Vrije Universiteit Medical Center, Amsterdam, NL. To evaluate CFU colony numbers in mouse experiments, Lin^{-} isolated cells from mice bone marrow were transduced using lenviral shRNA and seeded in 35 mm dishes at a density of 3×10^3 cells/well in triplicate in mouse MethoCult medium (M3434; Stem Cell Technologies). Hematopoietic colonies (CFUs) were counted at 7days. CFUs and average numbers of cells were counted for each of the three 35 mm dishes plated per sample and in each dishes, cells were counted and analyzed by flow cytometry. Data shown are mean colony or cells number per dishes ± SEM. Clonogenic activity of *Fancg*$^{-/-}$ or WT lin- cells transduced by shRNA in methylcellulose M3434 medium was tested. Colonies were scored and $9x10^3$ cells were replated in new M3434 medium every 7 days.

Flow Cytometry, Cell Sorting, and Purification

BM cells were resuspended at 10^8cells/mL and incubated with a lineage Lin^{-} cocktail 'biotin mouse lineage panel' against B220, GR-1, MAC-1, CD3, and TER119 (Ref: 559971, BD Biosciences, PharMingen) and progenitors enriched using Mylteni magnetic procedure. Flow cytometry analyses were performed by staining cells with an antibody cocktail containing anti-mouse C-KIT-PerCP5.5 (YB5.B8), GR-1 APC (RB6-8C5), MAC-1 PE (M1/70), TER119 PEcy7 (TER-119) and CD71 BV510 (C2)(BD Biosciences). Flow cytometric analysis and cell sorting were performed using a FACSCantoII Flow Cytometer (BD Biosciences) or a FACSAriaIII (BD Biosciences), respectively. Immunophenotypic data were analyzed sing the FlowJo Version 10 software (TreeStar).

Supplemental Figures

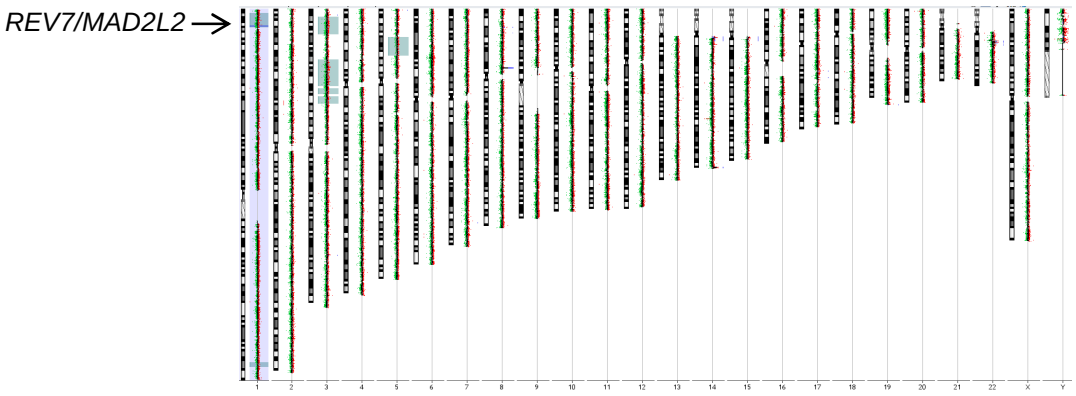

Figure S1
Homozygosity analysis of fibroblast gDNA of the patient EGF123 by SNP microarrays. Rare regions of homozygosity are detected (boxed in light green), which included the *REV7* gene (indicated by the arrow), suggesting distant consanguinity.

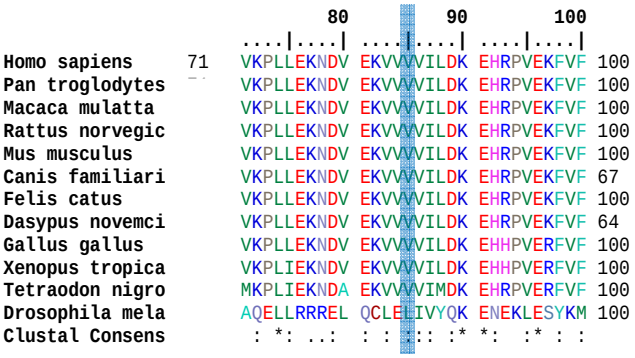

Figure S2
Schematic conservation of the HORMA domains of REV7; the Valine 85 residue is highlighted.

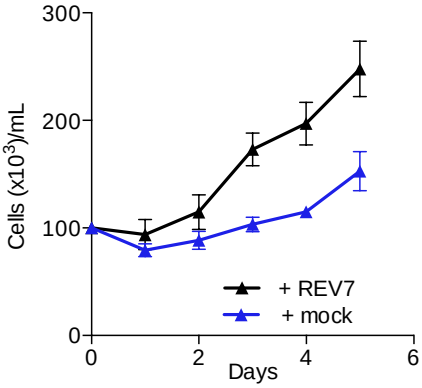

Figure S3
Spontaneous proliferation of EGF123 EBV-transformed cells, transduced with WT *REV7* or empty vector.

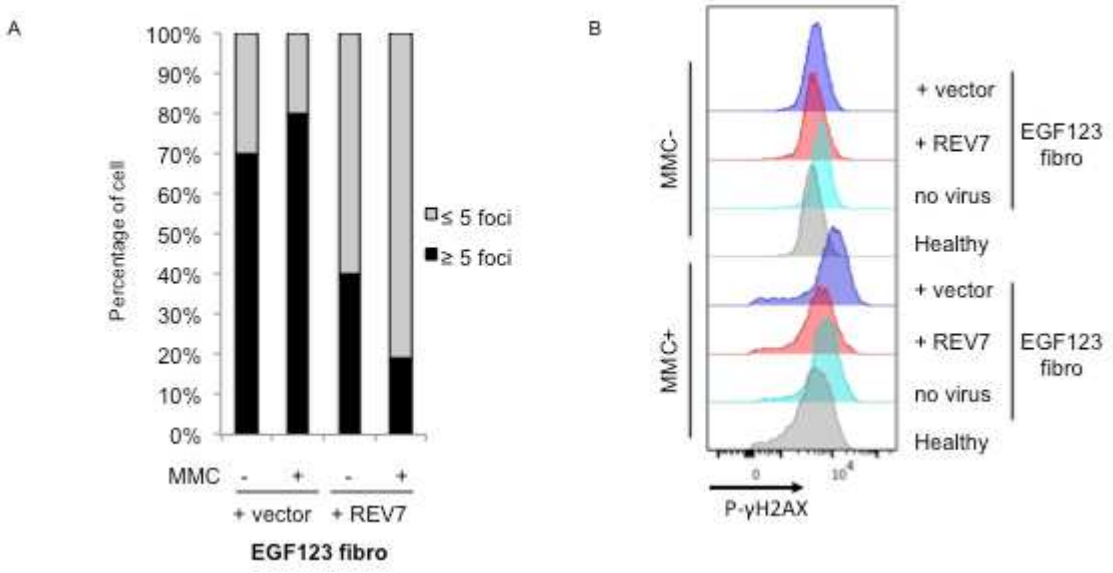

Figure S4
(A) The number of foci 53BP1 foci counted in fifty cells and the percentage of cell presenting more or less than 5 foci is shown in the graph. (B) DNA Damage detection using Flow cytometry detection of P-γH2AX achieved via tagging the protein with a fluorescently labelled antibody after treatment with BD CytoFix/Perm reagent. Experiments were performed using EGF123 primary fibroblasts transduced with *REV7* or empty vector or healthy fibroblasts, with or without MMC exposure.

Supplemental Table 1

Primer	Sequence 5'-3'	used for
ex5_F	TGGAGGCAGGGTGGTGTAGT	sanger sequencing of human REV7 exon5
ex5_R	TGCCAGAGACAACCTGATG	sanger sequencing of human REV7 exon5
hGADD45A_F_SYBR	CTGGAGGAAGTGCTCAGCAAAG	qRT_PCR
hGADD45A_R_SYBR	AGAGCCACATCTCTGTCGTCGT	qRT_PCR
hBAX_F_SYBR	TCAGGATGCGTCCACCAAGAAG	qRT_PCR
hBAX_R_SYBR	TGTGTCCAGCGGCAATCATC	qRT_PCR
hCDKN1A_F_SYBR	AGGTGGACCTGGAGACTCTCAG	qRT_PCR
hCDKN1A_R_SYBR	TCCTCTTGGAGAAGATCAGCCG	qRT_PCR
hMDM2 _F_SYBR	TGTTTGGCGTGCCAAGCTTCTC	qRT_PCR
hMDM2 _R_SYBR	CACAGATGTACCTGAGTCCGATG	qRT_PCR
HPRT_F_SYBR	GGCAGTATAATCCAAAGATGGTCAA	qRT_PCR
HPRT_R_SYBR	TCAAATCCAACAAAGTCTGGCTTATAT	qRT_PCR
ms_shREV7	GATCCCCCATCTTCCAGAAGCGCAAGAATTCAAGAGATTCTTGCGCTTCTGGAAGATGTTTTTGGAAA	cloning
MluI_REV7_F	GATACAACGCGTatgaccacgctcac	cloning
NheI_REV7_R	GTTAACGCTAGCtcagctgcctttatg	cloning

Table S1. Sequences of primers

Supplemental references

33 Bluteau D, Gilles L, Hilpert M, Antony-Debre I, James C, Debili N, Camara-Clayette V, Wagner-Ballon O, Cordette-Lagarde V, Robert T, et al. Down-regulation of the RUNX1-target gene NR4A3 contributes to hematopoiesis deregulation in familial platelet disorder/acute myelogenous leukemia. *Blood.* 2011;118(24):6310-20.

34. Soulier J, Leblanc T, Larghero J, Dastot H, Shimamura A, Guardiola P, Esperou H, Ferry C, Jubert C, Feugeas JP, et al. Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. *Blood.* 2005;105(3):1329-36.

35. Pinto FO, Leblanc T, Chamousset D, Le Roux G, Brethon B, Cassinat B, Larghero J, de Villartay JP, Stoppa-Lyonnet D, Baruchel A, et al. Diagnosis of Fanconi anemia in patients with bone marrow failure. *Haematologica.* 2009;94(4):487-95.