SUPPLEMENTARY INFORMATION

EXPERIMETAL PROCEDURES

Cell Culture: 4pX-1 cells were grown in DMEM/F12, pH 7.2 containing 2 mM L-glutamine, 0.24% sodium bicarbonate, 50 μg/ml gentamycin, 10% FBS, Insulin-Transferrin-Selenium (ITS), G418, and hygromycin were supplemented as described (40). Tetracycline (5 μg/ml) was added to block pX expression. HepG2, Hep3B and Huh7 were grown in DMEM supplemented with 10% FBS. SNU423 cells were maintained in RPMI 1640 supplemented with 10% FBS. HepAD38 were maintained in DMEM/F12 supplemented with 10% FBS, 100 μg/ml kanamycin, 300 ng/ml tetracycline and 400 μg/ml G418. The following reagents were used: nocodazole (300 ng/ml), Sigma; Doxorubicin (0.25-0.5 μg/ml) Calbiochem; BI 2536 (0.5 μM) AxonMedChem. Hep3B, Huh7, and SNU423 cell lines were purchased from ATCC. HepG2 and HepAD38 cells (40) were kindly provided by Drs. M. Bouchard and C. Seeger, respectively.

Flow Cytometry: cells were harvested following trypsin treatment, counted and centrifuged for one minute at 2000 rpm. 1ml PBS (phosphate-buffered-saline) was added to the pellet followed by centrifugation for one minute at 2000 rpm and removal of the supernatant. Cells were stored at -80°C until analyzed. For analysis, cells were incubated at 4°C for two hours in the presence of 50% Vindelov's reagent containing PBS, 3.5 units/ml RNase A, 75 mg/ml propidium iodide, 0.1% NP-40 and 50% IF buffer (130 mM NaCl, 3.5 mM NaH_2PO_4, 7.7 mM NaN_3). Unsynchronized 4pX-1 cells were used as control. Chick erythrocytes were added as an internal control and analyzed on Cytomics FC-500 (Beckman-Coulter) flow cytometer.

Live Cell Sorting: 4pX-1 cells were synchronized by serum starvation in DMEM/F12 containing 10μM EGFRi for 18 h (39). Serum starved cells were fed 10% FBS to re-enter the cell cycle. Tetracycline was removed at 10 h after addition of 10% FBS; nocodazole (250 ng/ml) was added for 6 h prior to cell harvesting. Cells were harvested by trypsin treatment, resuspended in Dulbecco's modified Eagle's medium/F-12 medium containing 20% FBS, and stained with Hoechst 33422 (500 ng/ml) 1 h before cell sorting. Cells were sorted for DNA content employing the Epics Altra cell sorter (Beckman-Coulter). Unsynchronized 4pX-1 cells were employed as control. Immediately after sorting, 100,000 cells containing >4N DNA content were plated in soft agar.

Soft Agar Assays: Tissue culture dishes (6 cm) coated with 1% tissue grade agarose (Bio-Rad) were seeded with 100,000 cells and growth was monitored for 15 days. 300μl of fresh medium was added every three days. Colonies were imaged using Nikon TE300 at 10X magnification. Colonies were quantified by Image J software.

Real-Time PCR: cDNA synthesis was performed with 2 μg total RNA using iSCRIPT cDNA synthesis kit (Bio-Rad). Real-Time PCR reactions were performed using the protocol provided by manufacturer (Applied Biosystems). Reactions were normalized to GAPDH. PCR reactions were carried out in triplicate using ABI PRISM 7700 System (Perkin-Elmer).

SUZ12 primers

Fwd: 5'-AAAACTCTGAGAACTTGCCCC-3'

Rev: 5'-ACACTGCCTGTTCCCAATCC-3'

ZNF198 primers

Fwd: 5'-GAAGGGAGCCACTAAAGAAC-3'

Rev: 5'-GAGTGCCTTGGGATTTACAG-3'

Immunofluorescence Microscopy for PML: Cells were fixed in 4% paraformaldehyde (Sigma) or 75% ethanol for 20 min, washed three times in PBS, and incubated for 1 h in blocking buffer containing 130 mM NaCl, 7 mM Na_2HPO_4, 3.5 mM NaH_2PO_4, 7.7 mM NaN_3, 0.1% bovine serum albumin, 1.0% Triton-X-100, 0.05% Tween 20, and 10% goat serum. Incubation with primary antibody was carried out at 4°C at the following dilutions: 1:200 for PML (Santa Cruz). Incubation with secondary antibodies AlexaFluor488 (green) and AlexaFluor568 (red), both from Invitrogen, was for 1 h at room temperature. Cells were visualized by Nikon TE300 at 20X and 60X magnification.

Preparation of Whole Cell Extract (WCE) and Immunoblotting: Cells were harvested in RIPA buffer containing 50 mM Tris pH7.4, 150 nM NaCl, 1% NP-40, 1 mM EDTA, 100 mM NaF, 50 mM glycerol phosphate, 1 mM sodium orthovanate, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg /ml leupeptin, 1 μg/ml pepstatin, and sonicated on ice for 10 seconds. Lysates were clarified by centrifugation at 12,000 rpm for 15 minutes at 4°C. Protein concentration was determined by the Bio-Rad protein assay. 20 μg WCE were boiled in 2X SDS-PAGE loading buffer (4% SDS, 20% glycerol, 2% β-mercaptoethanol, 160 mM Tris pH 6.8), electrophoresed on SDS-PAGE and transferred to nitrocellulose membrane. Primary antibodies: Plk1 1:1000 (abcam), SUZ12 1:1000 (abcam), ZNF198, 1:500 (Bethyl), PML 1:500 (Santa Cruz) p53 1:1000 (Vector Laboratories), Cleaved Caspase 3 1:1000 (Cell Signaling), γ-H2AX 1:2000 (Calbiochem)

HBV Replication Assay: HepAD38 cells were grown in 6 cm tissue culture dishes, for indicated time course (0-20days) with or without tetracycline (5µg/ml). Cells were harvested in 0.2 ml of tissue lysis buffer (50 mM Tris, 0.5% NP-40, 1 mM EDTA, 100 mM NaCl) and incubated at 4°C. Cell debris was pelleted and supernatant transferred to a new tube containing 2.0 µl of 1M $MgCl_2$ and 2.0 µl DNase 1. Mixture was incubated at 37°C for 2 h. DNase removal reagent (20 µl, Ambion) was used at room temperature, and pelleted after 2 min. incubation. The supernatant was used for isolation of HBV DNA from intracellular viral particles employing QIAamp MinElute Virus Spin Kit (Qiagen) according to manufacturer's protocol. Eluted DNA, 1.0 µl was used as template in real- time PCR reactions with HBV primers described by (43). Plasmid DNA carrying the HBV genome (ayw1.2) was serially diluted and used as quantification control, employing the same primers.

HBV primers:

Fwd: 5'-AGAAACAACACATAGCGCCTCAT-3'

Rev: 3'-TGCCCCATGCTGTAGATCTTG-3'

HBV Genome Equivalents were calculated using the following equation:

$$\text{NUMBER OF DNA COPIES} = \frac{(\text{Amount of DNA in nanograms} \times 6.022\times10^{23})}{(\text{Length} \times 10^{9} \times 650)}$$

The calculation makes the assumption that the average mass of a base pair is 650 Daltons. 6.022×10^{23} is Avogadro's number. The amount of DNA in nanograms is determined from the standard curve generated by serial dilution of the control HBV plasmid.

siRNA Transfection of HepAD38 cells: HepAD38 cells grown in the absence of antibiotics for 24 h were transfected using Lipofectamine 2000 (Invitrogen) according to manufacturer's protocol. The combination of three siRNAs for ZNF198, SUZ12 and PML was used at final

concentration of 33nM. Antibiotics including tetracycline were added for an additional 24 h, at 4 h after transfection. HBV replication was assayed as described earlier, using intracellular virions isolated from HepAD38 cells at 1, 5 and 10 days after tetracycline removal.

siRNA sequences:

PML siRNAs

Fwd: 5' GCCUGUCGGUGUACCGGCAGAUUGU 3'

Rev: 5' ACAAUCUGCCGGUACACCGACAGGC 3'

Fwd: 5' CAGGAGGUGCUGGACAUGCACGGUU 3'

Rev: 5' AACCGUGCAUGUCCAGCACCUCCUG 3'

Fwd: 5' UCGACGAGUUCAAGGUGCGCCUGCA 3'

Rev: 5' UGCAGGCGCACCUUGAACUCGGCGA 3'

SUZ12 siRNAs

Fwd: 5' GCCGCAAACUUUAUAGUUUACUCAA 3'

Rev: 5' UUGAGUAAACUAUAAAGUUUGCGGC 3'

Fwd: 5' CCUAUGCAGGAAAUCCUCAGGAUAU 3'

Rev: 5' AUAUCCUGAGGAUUUCCUGCAUAGG 3'

Fwd: 5' CCAUGUCAUGAAGCAUGGGUUUAUU 3'

Rev: 5' AAUAAACCCAUGCUUCAUGACAUGG 3'

ZNF198 siRNAs

Fwd: 5' UCGCCAGUUUGUAGCGCCAAGUGAU 3'

Rev: 5' AUCACUUGGCGCUACAAACUGGCCA 3'

Fwd: 5' GCAACUAUUGUUCUCAGCUAUGUAA 3'

Rev: 5' UAACAUAGCUAGAACAAUAGUUGC 3'

Fwd: 5' UUUAGUUGCUCCCUUCUUACAUAGC 3'

Rev: 5' GCUAUGUAAGAAGGGAGCAACUAAA 3'

FIGURE LEGENDS

Supplementary fig. 1. Identification of genes whose protein depletion rescued pX-expressing 4pX-1 cells from DNA damage-induced apoptosis. The tetracycline-regulated pX-expressing 4pX-1 cell line (39), an immortalized, less-differentiated hepatocyte cell line (14) was used for this study. We generated a stable population of 4pX-1 cells infected with a lentiviral shRNA library. Infected 4pX-1 cells were grown for 10 days in apoptotic conditions induced by pX + doxorubicin. Cells surviving apoptosis for 10 days were propagated separately, generating individual cell clones. The shRNA insert of surviving clones was sequenced and the depleted gene identified. The most frequent genes detected by this screen were ZNF198 and SUZ12. *Topors (43) was identified by a similar screen, involving 10-day selection of infected 4pX-1 cultures with pX + doxorubicin, but without propagation of surviving cells.

Supplementary fig. 2. ZNF198 and SUZ12 associate with PML NBs in pX-expressing cells. **A.**

Lysates from 4pX-1 cells grown ± pX and ± doxorubicin treatment, as indicated, were immunoprecipitated with PML antibody. IgG immunoprecipitations with lysates from 4pX-1 cells expressing pX. PML and IgG immunoprecipitates were immunoblotted for the indicated proteins. **B.** Confocal microscopy of 4pX-1 cells with indicated antibodies.

Supplementary fig. 3: . Knockdown of ZNF198 and SUZ12 increases cell survival in conditions of pX-mediated apoptosis.**.** Lysates from indicated cell lines grown without (-) pX and in the presence of doxorubicin (0.5 μg/ml) for 6 h . Actin is loading control.

Supplementary fig. 4. Knockdown of ZNF198 and SUZ12 increases cell survival in conditions of pX-mediated apoptosis. **A.** Immunoblots of ZNF198, SUZ12, Topors and PML using lysates from clonal isolate-2 of indicated knockdown cell lines. 4pX-1 cells with stable integration of pGIPZ empty vector were constructed in parallel. **B.** Immunoblots of p53 using lysates from indicated clonal isolate-2. Cells were grown ± pX and doxorubicin (0.5 μg/ml) for 6 h.

Supplementary fig. 5: Defective DNA repair in ZNF198 and SUZ12 knockdown cells
A. Comet assay of cells grown ± pX for 24 h. Cells stained with SYBR Green were analyzed by agarose gel electophoresis (13). **B.** Quantification of cells with DNA tailing. (250 cells counted, from three independent assays). **C.** Quantification of length of DNA tails using Comet Assay IV software, Perspective Instruments. **D.** Immunoblots of γ-H2AX using lysates from indicated cell lines grown ± pX for 24 h.

Supplementary fig. 6. Immunoblots of ZNF198, SUZ12 and p53 using WCE isolated from the

indicated cell lines after time course treatment (0-12 h) with 0.5 μg/ml doxorubicin. Actin is the loading control. The status of endogenous p53 is indicated in parenthesis.

Supplementary fig. 7. Immunofluorescence microscopy of endogenous PML or transfected PML-RFP protein in 4pX-1, 4pX-1-SUZ12^{kd}, 4pX-1-ZNF198^{kd,} and 4pX-1-PML^{kd} at 60X magnification. Quantification of PML NBs is from approximately 100 cells.