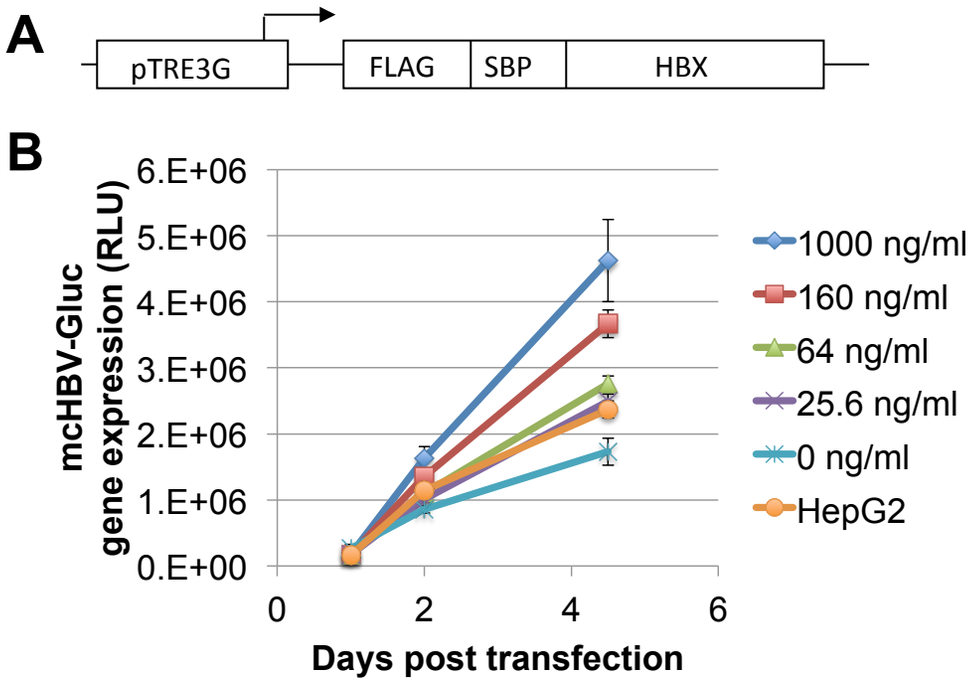
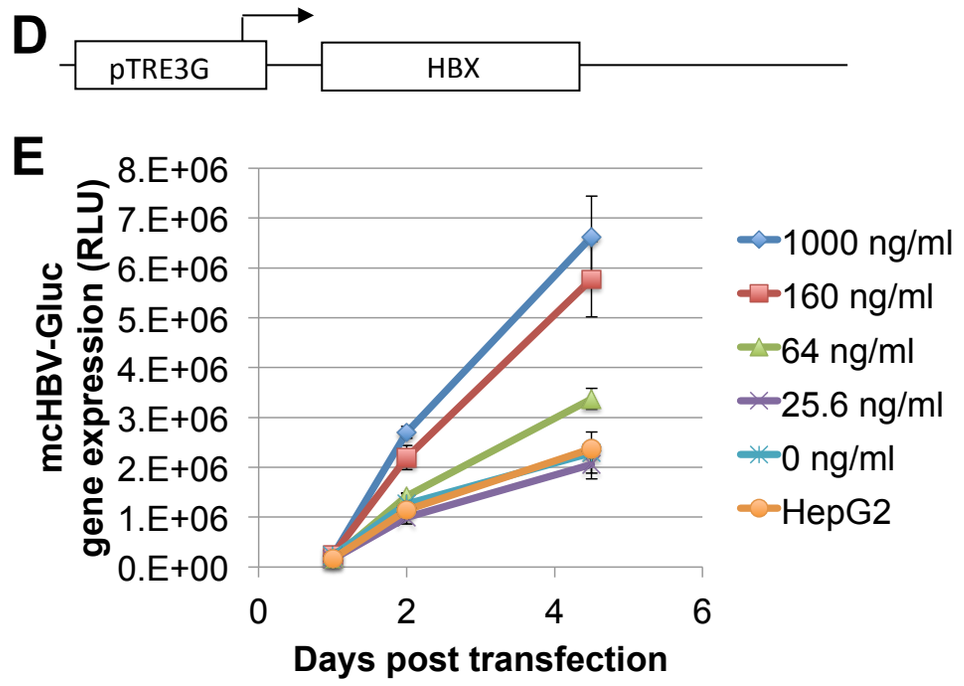
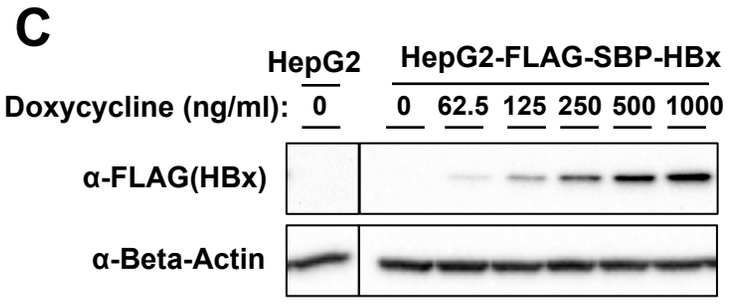
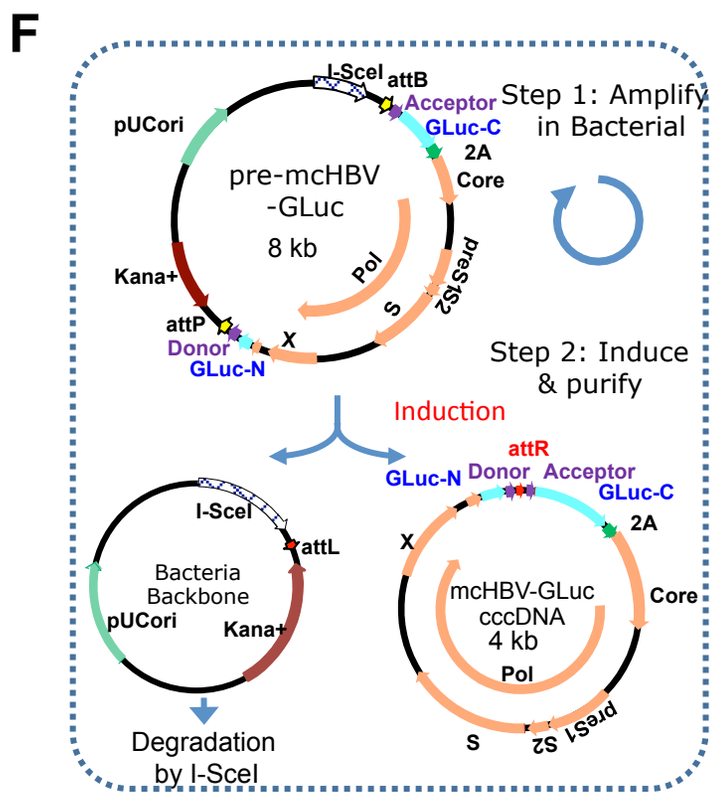
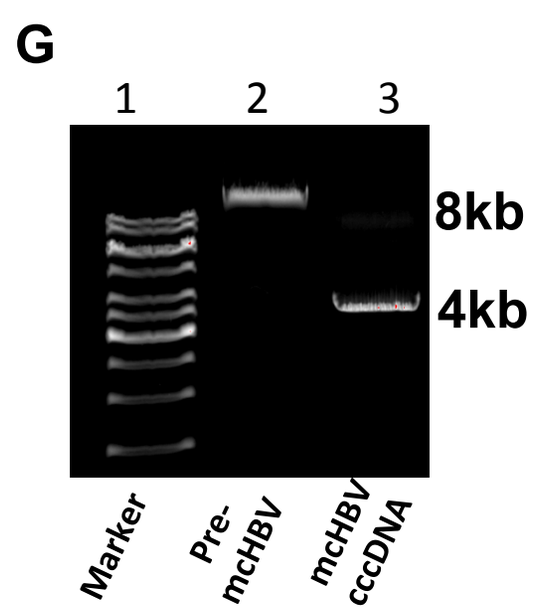
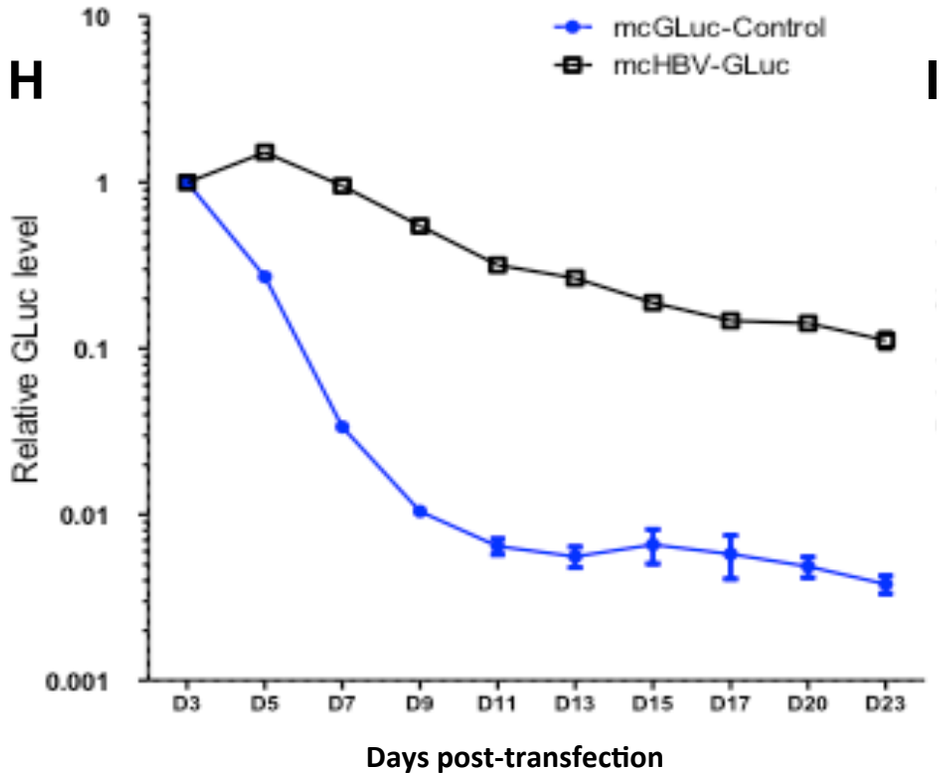


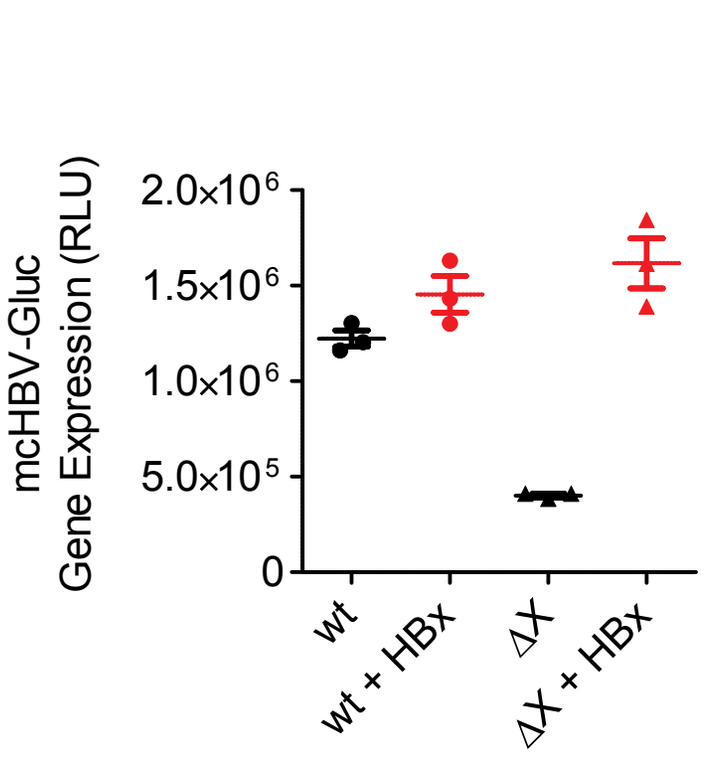




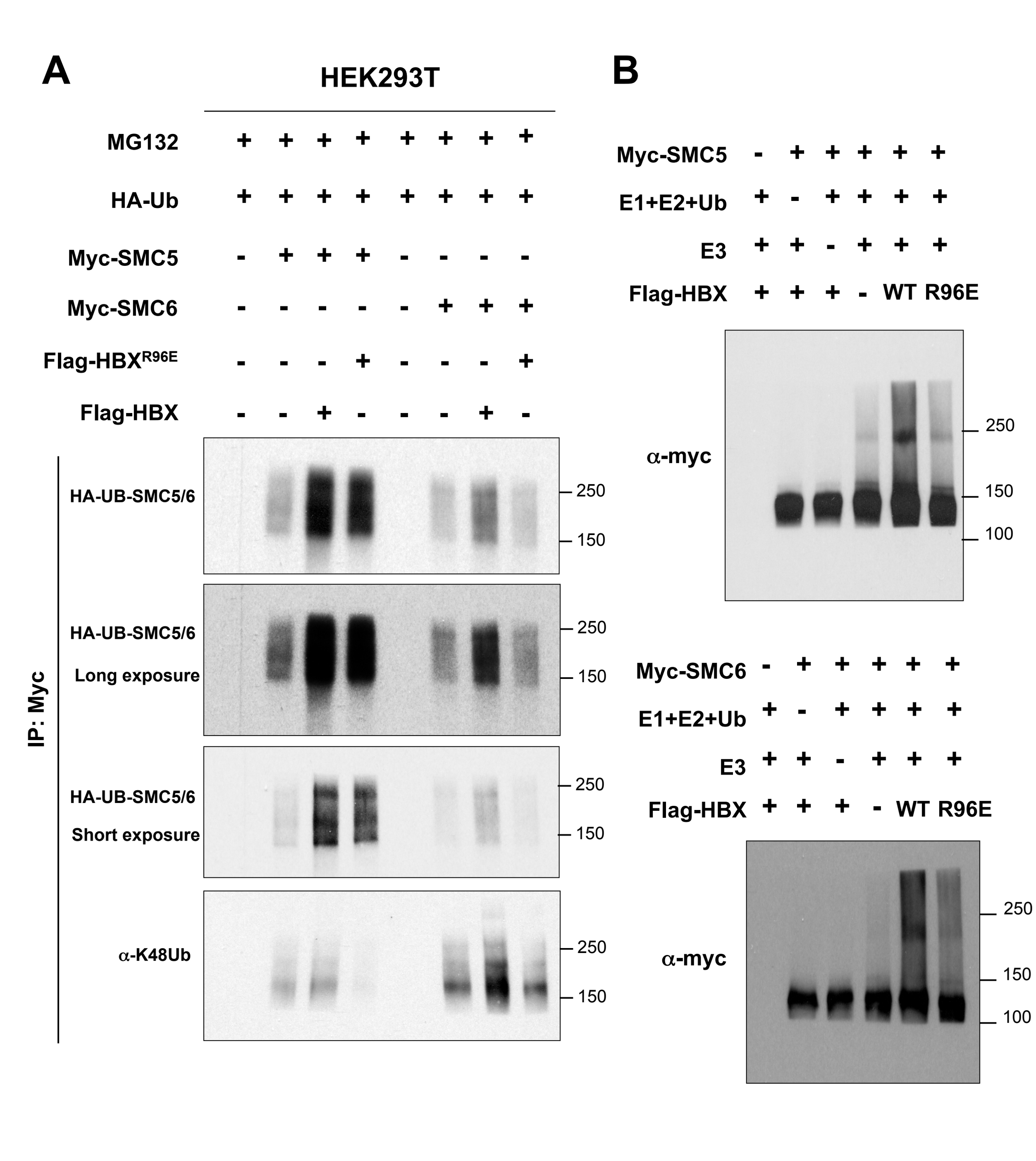








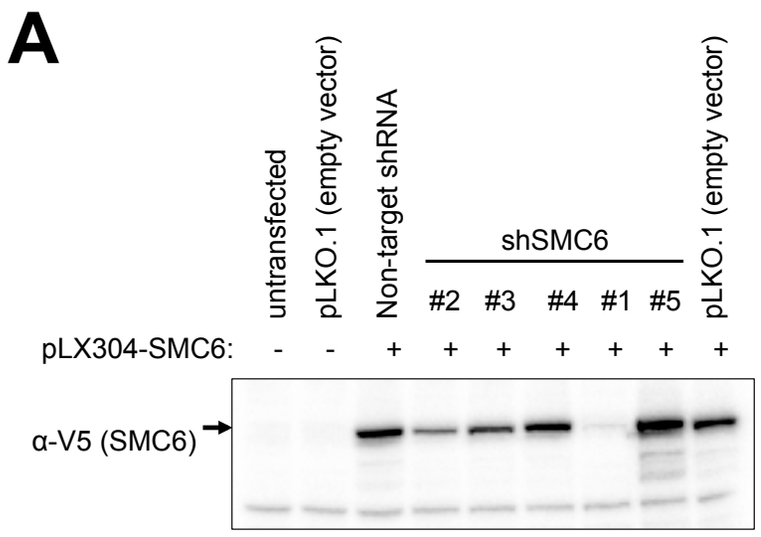
Supplementary Figure S1. Experimental systems. Related to Figures1 & 4. A-E. Inducible HepG2-HBx cell lines. Stable HepG2 cell lines were created that express FLAG-SBP-HBx (HepG2-HBx-FSH8; A) or untagged HBx (HepG2-HBx-H5; D) under the control of the tetracycline-responsive TRE3G promoter, and HBx function was measured across a range of doxycycline concentrations by transfecting minicircle HBV-Gluc cccDNA and monitoring luciferase in the culture media (B and E, respectively). FLAG-SBP-HBx expression was monitored at a range of doxycycline concentrations (C)**. F-I.** Minicircle cccDNA HBV reporter system**.** (F) Schematic of minicircle cccDNA production and structure. (G) Efficient recombination of minicircle HBV-Gluc cccDNA after induction in bacteria. (H) Stable expression of luciferase from mcHBV-Gluc cccDNA after transfection in human hepatocytes. A minicircle DNA encoding Gluc under control of the EF1a promoter was used as a control. I) Full cccDNA activity is dependent upon HBx activity. Minicircle cccDNA, either wild type or HBx-null, were transfected into inducible HepG2-HBx-H5 cells. Doxycycline was added to induce HBx expression. Luciferase activity in culture media was measured after 7 days.

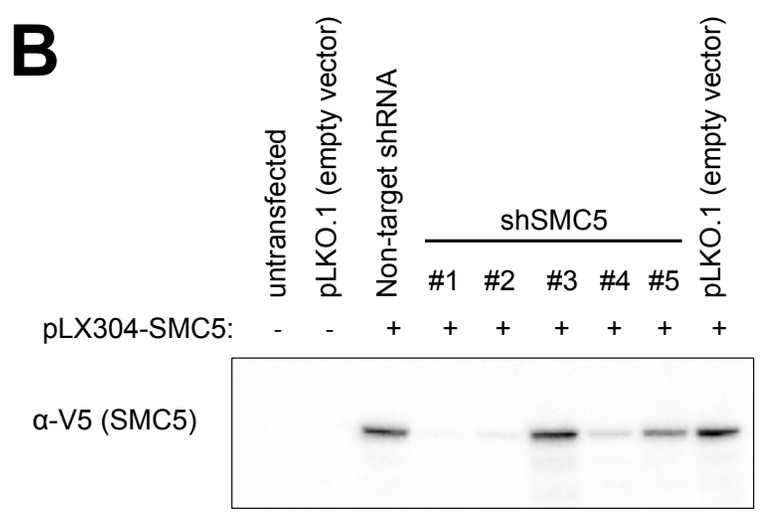


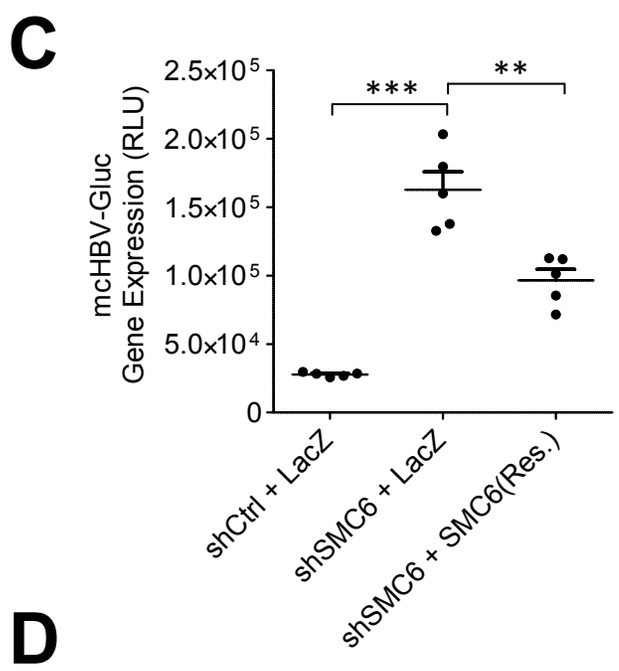
Supplementary Figure S2. HBx targets SMC5/6 for the ubiquitylation by CRL4 E3 ligase. Related to Figure 3

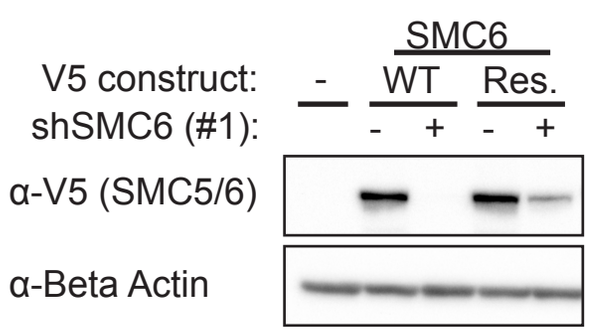
A. Different exposure of ubiquitylated SMC5/6 proteins, related to Figure 3A.

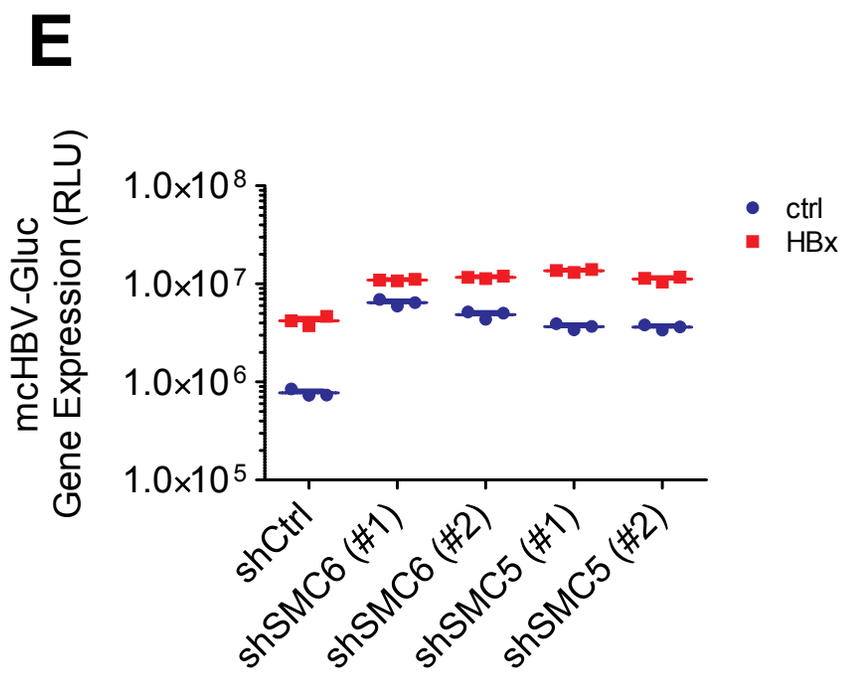
B. Long exposure of anti-myc antibody. The strong band around maker 150kDa is the un-ubiquitylated SMC5/6, the smear above is the ubiquitylated SMC5/6, related to Figure 3C/D.

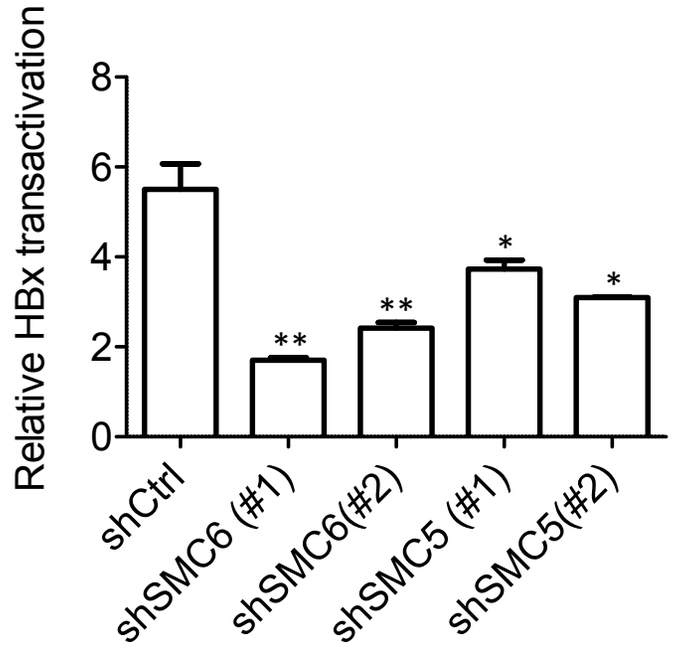


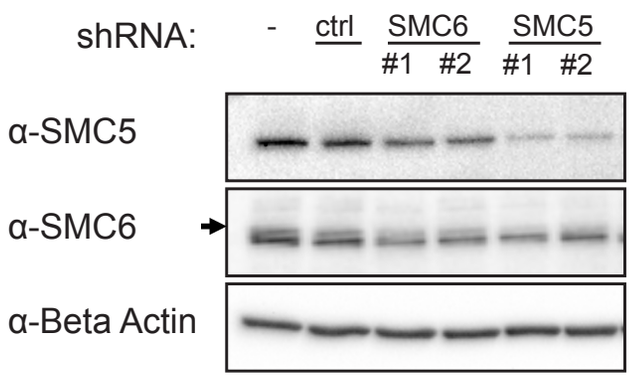




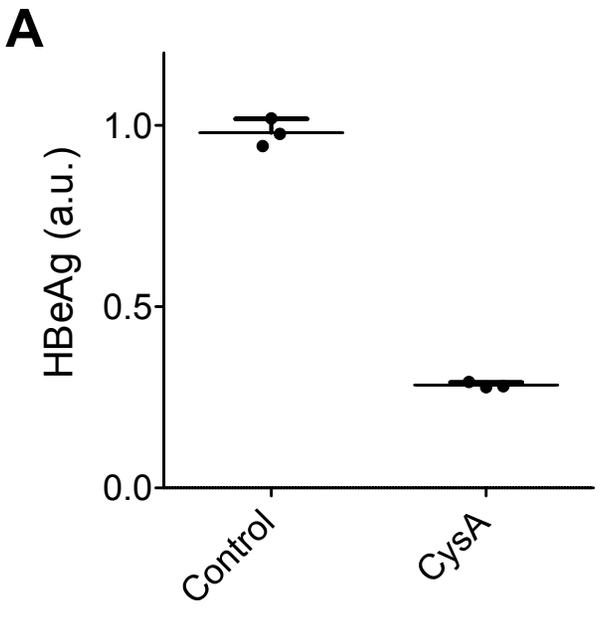


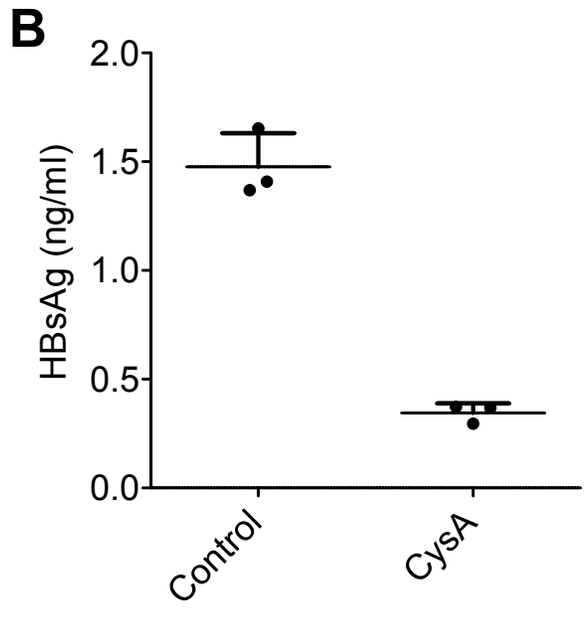


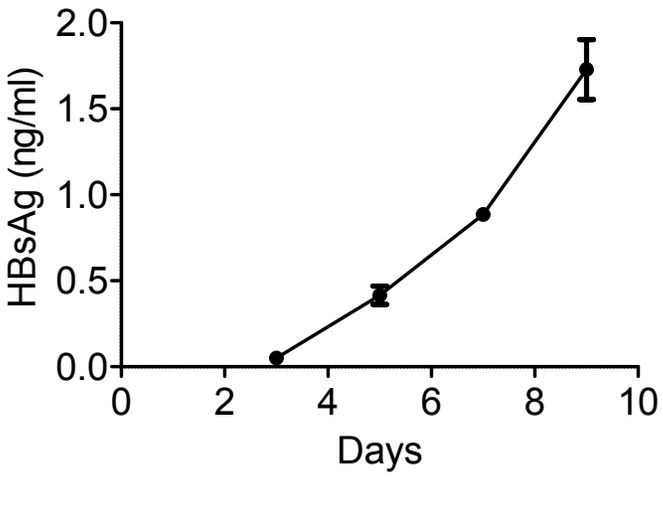


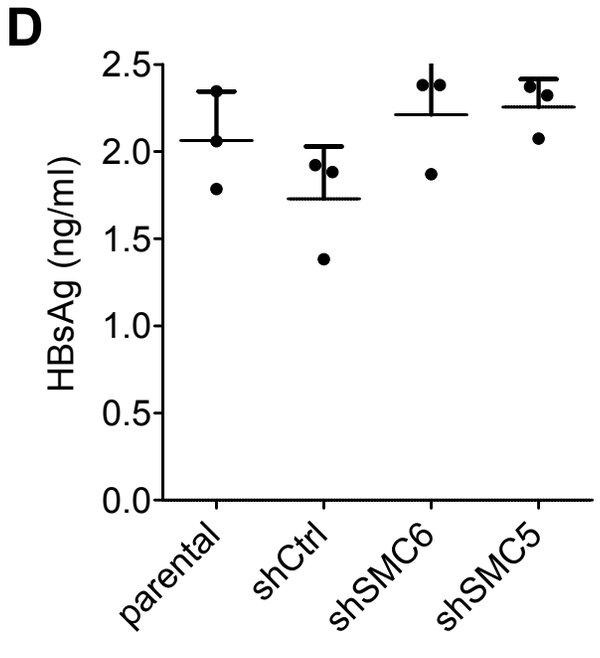


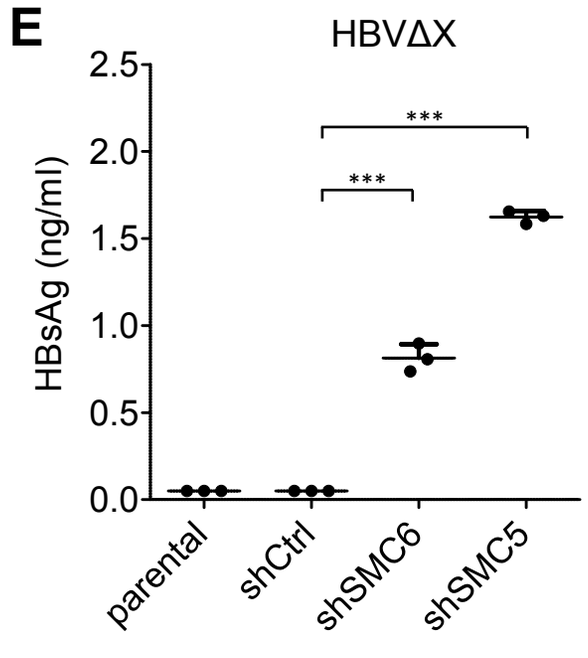
Supplementary Figure S3. Knockdown of SMC5/6 and its effect on HBx transactivation. Related to Figure 4. A-B. The effectiveness of shRNAs targeting SMC6 or SMC5 was determined by co-transfection with SMC6 or SMC5 expression constructs. **C.** Restoration of cccDNA inhibition by SMC6**.** shRNA–resistant SMC6 was cotransfected together with mcHBVΔX cccDNA into HepG2 cells. SMC6(Res) indicates SMC6 bearing silent mutations in the shRNA target sequence. Luciferase was assessed after 8 days. **D.** HEK293T cells were co-transfected with SMC6 expression constructs along with shRNA targeting SMC6 (#1). "Res." indicates an shRNA resistant form of SMC6. **E.** HBx transactivation is curtailed in cells with SMC5/6 knockdown. HBx was induced in a second set of samples from the experiment in Figure 4A. Luciferase was measured after 14 Days. **F.** Fold transactivation by HBx was calculated for the samples in E. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. **G.** Cells from the experiment in E and Figure 4A were collected after 21 days and analyzed for SMC5/6 levels. Arrows indicate the correct bands.

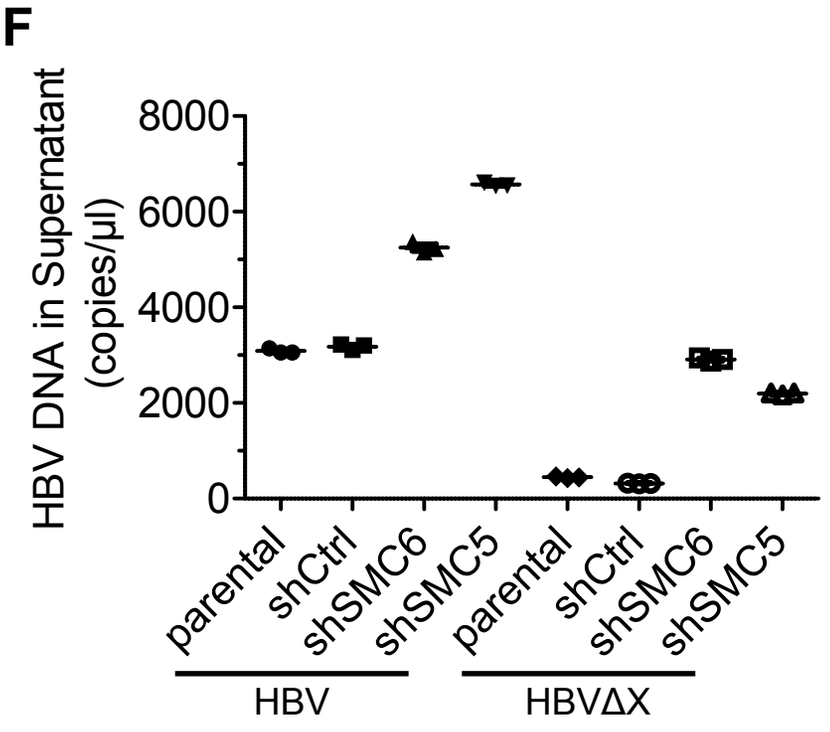


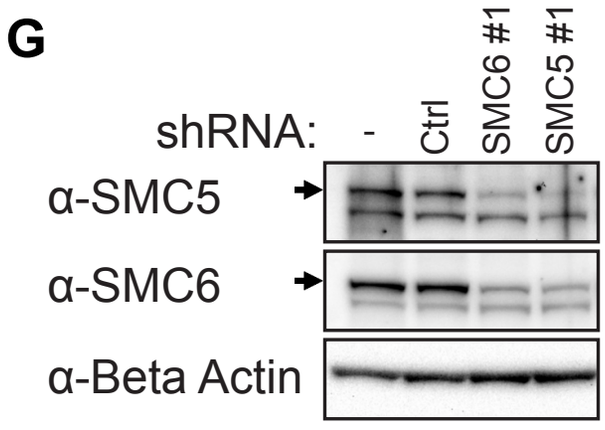












Supplementary Figure 4. HBV infection of HepG2-NTCP cells. Related to Figure 4. A-B. Treatment with the HBV entry inhibitor cyclosporin A (CysA, 8μM) blocked subsequent release of HBeAg and HBsAg at 9 days post infection. **C**. HepG2-NTCP cells were infected with HBV, and HBsAg release began several days later. **D-E.** An additional HBV infection experiment, similar to Figure 4E, is shown. HepG2-NTCP cells stably expressing shRNA targeting either SMC6, SMC5, or a control sequence were infected with wt HBV (D) or HBVΔX (E), and HBsAg levels in the culture media were assessed by ELISA after 9 days. **F.** Viral DNA was isolated from culture media from the experiment in Figure 4C-F, and HBV genomes were quantified by qPCR. **G.** SMC5/6 levels were assessed by western blot in HepG2-NTCP cells stably expressing shRNA targeting SMC5 or 6.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture, transfection, and transduction

HEK293T, HepG2, TetOn Advance HepG2 (Clontech), and HepG2-NTCP cells were grown in DMEM with 10% FBS, penicillin, and streptomycin. Inducible HBx cell lines were constructed by transfecting pTRE3G-HBx (HepG2-HBx-H5) or pTRE3G-FLAG-SBP-HBx (HepG2-HBx-FSH8) into TetOn Advance HepG2 (Clontech). Individual colonies were tested for inducible HBx expression. Transfections were performed using XtremeGENE HP (GE Life Sciences) for HEK293T cells or Lipofectamine 3000 (Life Technologies) for HepG2. Stable SMC5/6 knockdown cells were established by transduction of HepG2-NTCP cells with the appropriate lentivirus at MOI <1, followed by selection with 1.4 ug/ml puromycin.

Expression Constructs.

HBV sequences were cloned from patient-derived genotype C HBV. The HBx open reading frame was cloned into p3XFLAG-CMV-7.1 (Sigma). A FLAG-SBP-HBx sequence containing an 8 amino acid glycine linker between SBP and HBx was cloned into pTRE3G (Clontech). Minicircle HBV cccDNA reporter system was described in Supplementary Figure 1.. A minicircle DNA encoding Gluc under control of the EF1a promoter was used as a control. SMC5 and SMC6 open reading frames were cloned into pLX304 using Gateway cloning. HBx-deficient minicircle cccDNA was created by introducing premature stop codons into the 8th and 87th amino acid positions, but without affecting the pol coding sequence (Cha et al., 2009). shRNA constructs for SMC6 (TRCN0000219949, #1, and TRCN0000183231,

#2) and non-target control (SHC016) were obtained from Sigma. SMC5 shRNA constructs (TRCN0000148162, #1, and TRCN0000147948, #2) were obtained from the UNC Lentiviral Core.

Antibodies

Antibodies against SMC6 were obtained from Abcam (18039), Santa Cruz Biotech (sc-365742), and Abgent (AT3956a). SMC5 antibodies were obtained from Genetex (gtx115669) and Abcam (18038). The following antibodies were obtained from One World Lab: Fbl (A-1136), RALY (AP16333c), BUB1 (A1929), DHX9 (A300-854A), DDX5 (A300-523A), NLRC4 (AP13307a), NSE4A (AP9909a), RBL1(PA1894), and NDNL2 (ESAP16578). THADA (Genetex GTX104909), V5-HRP (Life Technologies), beta-actin-HRP (Abcam), Flag-HRP (Sigma), CUL4B (Sigma HPA011880), HBs (SantaCruz sc-53299), HBc (Zeta Z2085), and HBx (Biovendor RD981038100) antibodies were also used.

Ubiquitylation assays

For in vitro ubiquitylation assay, CUL4-DDB1 immune complexes were purified from HEK293T cells transfected with Myc-CUL4A and Myc-CUL4B with anti-Myc antibody (clone 9E10) and Protein G (Invitrogen) in NP-40 lysis buffer (0.3% Nonidet P-40, 50 mM Tris pH 7.5, 150 mM NaCl). Immobilized Myc-CUL4A and Myc-CUL4B complexes were eluted with Myc antigen peptides. SMC5 and SMC6 were similarly purified from HEK293T cells. Ubiquitylation reactions were performed in a 50 μL reaction volume, containing 100 nM E1 (Enzo Life Sci.), 1 μM E2 UbcH5c (Enzo Life Sci.), 1 μM human recombinant HA-ubiquitin (Boston Biochem), 1 unit

inorganic pyrophosphatase, 1 mM DTT and 5 mM Mg-ATP, 100 ng of eluted CUL4 complexes and HBx protein as the source of E3 and 100 ng of human Myc-SMC5 or SMC6 as substrate. Reactions were incubated at 37 °C for 30 min, terminated by equal volume of SDS-PAGE buffer.

For the in vivo ubiquitylation assay, HEK293T cells were transfected with indicated plasmids and siRNA, and were treated with MG132 (20 μM) for 5 h. Cells were lysed under denaturing conditions in an SDS buffer (50 mMTris-HCl, pH 7.5, 0.5 mM EDTA, 1 mM DTT, 1% SDS) by boiling for 10min. Lysate was clarified by centrifugation at 13,000 rpm for 10 min and diluted 10-fold with an NP-40 buffer (50mM Tris pH 7.5, 150mM NaCl, 0.3% Nonidet P-40) and then subjected to immunoprecipitation using an anti-Myc antibody (9E10) followed by SDS-PAGE. Ubiquitylated SMC5 and SMC6 was detected with HA antibody.

Humanized mice, liver tissue preparation

Liver tissue from HBV-infected NRG-FAH humanized liver mice was prepared as reported (Li et al., 2014; Li et al., 2015). Liver tissue was homogenized in RIPA buffer with protease inhibitors, and clarified by centrifugation at 17,000 x g for 20 minutes. Supernatants were used for Western blots.