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Supplementary Materials for

Restriction of hepatitis B virus replication by c-Abl–induced proteasomal degradation of the viral polymerase

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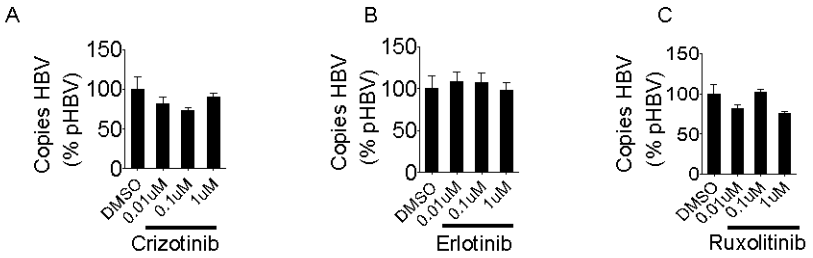

Fig. S1. Capsid-associated viral DNA level in the treatment of TKIs.

(**A**-**C**) Same experiment as in **Fig. 1A** but treated with crizotinib (**A**) (n=4 per group), erlotinib (**B**) (n=3-4 per group) and ruxolitinib (**C**) (n=3 per group), respectively.

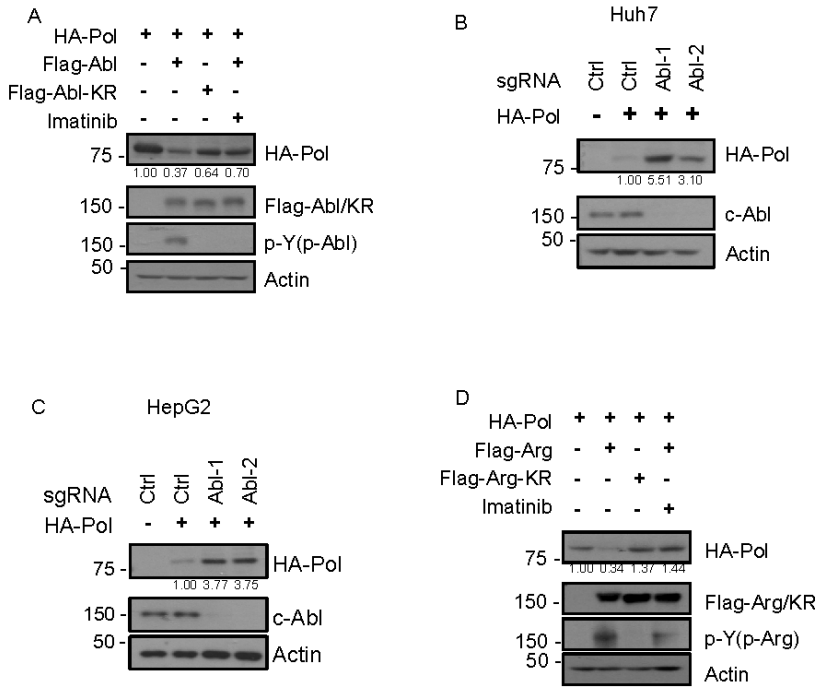

Fig. S2. c-Abl kinase reduces the level of HBV polymerase.

(**A**) Same experiment as in **Fig. 1G** but with HEK293T cells. (**B-C**) Immunoblot for polymerase protein level in WT or c-Abl KO Huh7 cells (**B**) or HepG2 cells (**C**). Total cell lysates were lysed 48 hrs after transfection and subjected to WB. (**D**) HEK293T cells were transfected with constructs expressing HA-polymerase, Flag-Arg, Flag-Arg-KR or empty vector control. After 24 hrs, cells were treated, or not treated, with 2 μM imatinib and harvested 24 hrs later. Total cell lysates were then analyzed for the indicated proteins.

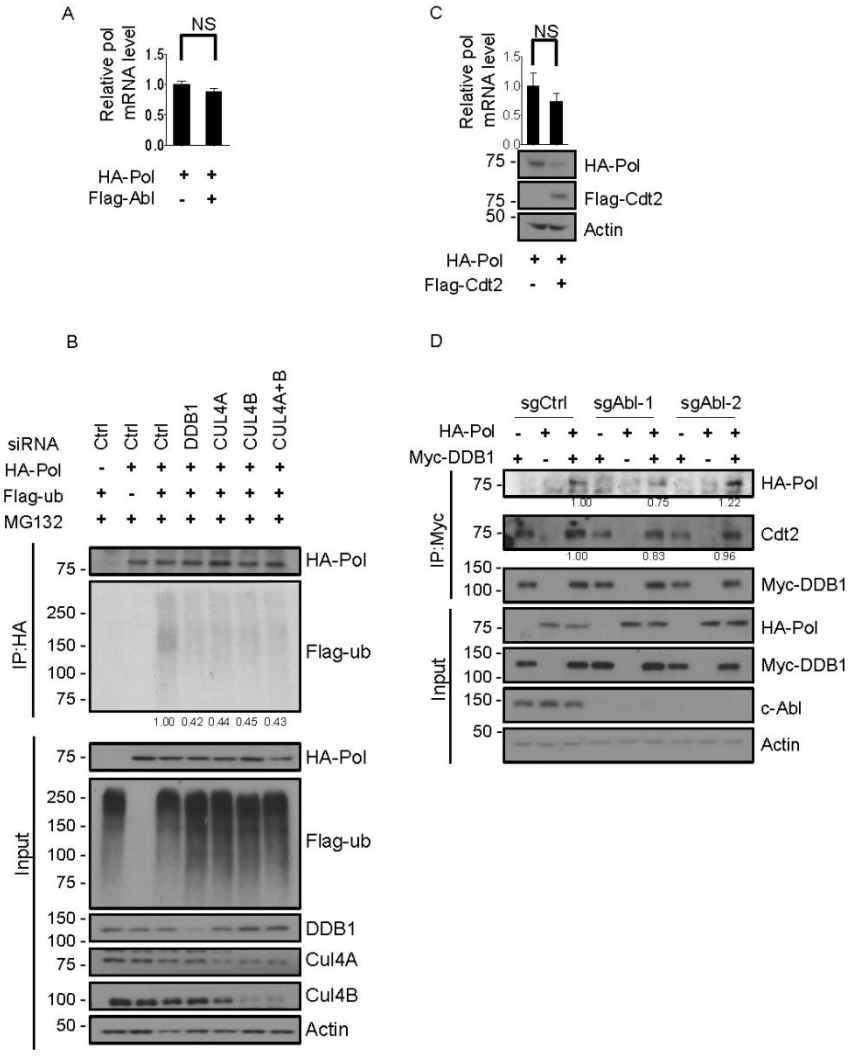

Fig. S3. c-Abl–CRL4^{Cdt2} reduces HBV polymerase by promoting its ubiqutination but not transcription level.

(**A**) Quantitation of polymerase mRNA by real-time PCR. mRNA purified from transfected HEK293T cells were diluted and the relative polymerase mRNA level without co-transfected Flag-Abl was set to 100% and compared to others. Statistical significance is indicated by asterisks above the brackets (n=8-10 per group). (**B**) Ubiquitination of polymerase by IP HA in Huh7 cells expressing HA-polymerase, Flag-ub and siDDB1, siCUL4A, siCUL4B or a combination of siCUL4A and

siCUL4B and treated with MG132 for 8 hrs. HA immunoprecipitates (top) or total cell lysates (bottom) were then analyzed by western blot. (**C**) Quantitation of polymerase mRNA by real-time PCR. mRNA purified from transfected HEK293T cells were diluted and analyzed by real-time PCR (top). The relative polymerase mRNA level without co-transfected Flag-Abl was set to 100% and compared to others (n=3-4 per group). Whole-cell lysates were prepared to subject to WB (bottom). (**D**) Co-immunoprecipitation of Myc-DDB1 with HA-polymerase and Cdt2 in WT or c-Abl KO HepG2 cells over-expressing HA-polymerase and Myc-DDB1.

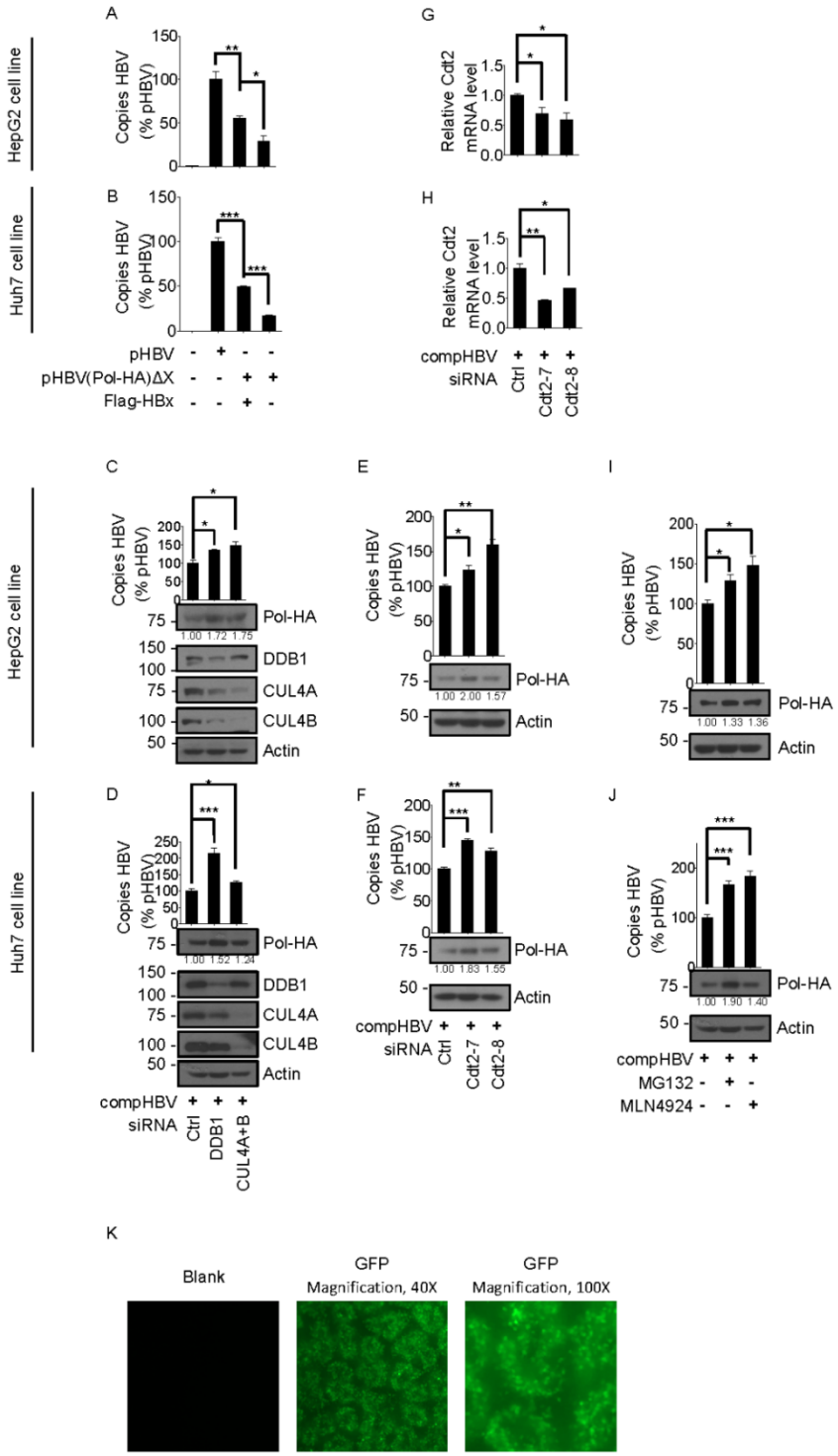

Fig. S4. Inhibition of CRL4 E3 ubiquitin ligase enhances HBV replication by stabilizing viral polymerase.

(**A**) HepG2 cells and (**B**) Huh7 cells were co-transfected with plasmids expressing pHBV, pHBV(Pol-HA)ΔX and HBx, and capsid-associated viral DNA were quantitated. Mean copy number from cells transfected with pHBV was set to 100% and compared to others (n=3-4 per group). (**C**) HepG2 cells were and (**D**) Huh7 cells transfected with indicated siRNAs and compHBV. Whole-cell lysates were prepared to subject to WB (bottom) and capsid-associated viral DNA were quantitated (top). Mean copy number from cells transfected with control siRNA was set to 100% and compared to others (n=3-4 per group). (**E**) HepG2 cells and (**F**) Huh7 were transfected with siCdt2 and compHBV. Whole-cell lysates were prepared to subject to WB (bottom) and capsid-associated viral DNA were quantitated (top). Mean copy number from cells transfected with control siRNA was set to 100% and compared to others (n=3-4 per group). (**G**, **H**) Real-time PCR assay to determine the efficiency of Cdt2 knockdown in HepG2 cells (**G**) and Huh7 cells (**H**) (n=3-4 per group). (**I**) HepG2 cells and (**J**) Huh7 cells were transfected with compHBV and treated with DMSO, MG132 or MLN4924. Whole-cell lysates were prepared to subject to WB (bottom) and capsid-associated viral DNA were quantitated (top). Mean copy number from cells treated DMSO was set to 100% and compared to others (n=3-4 per group). (**K**) Detection of GFP in fresh liver tissue sections from the indicated mice hydrodynamic tail vein injected into GFP or not.

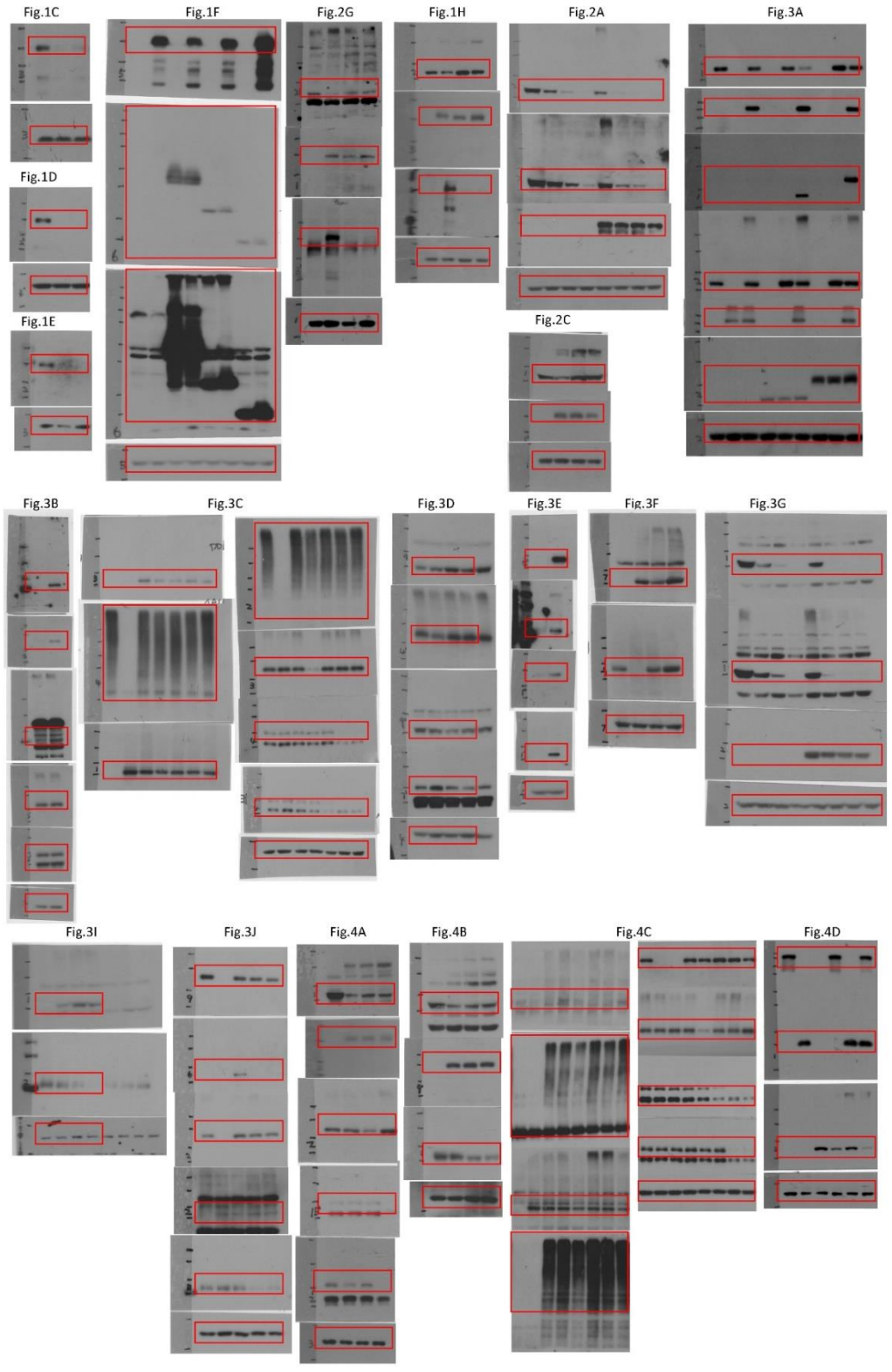

Fig. S5. Western blot–scanned films. Red boxes highlight lanes used in Fig. 1-4

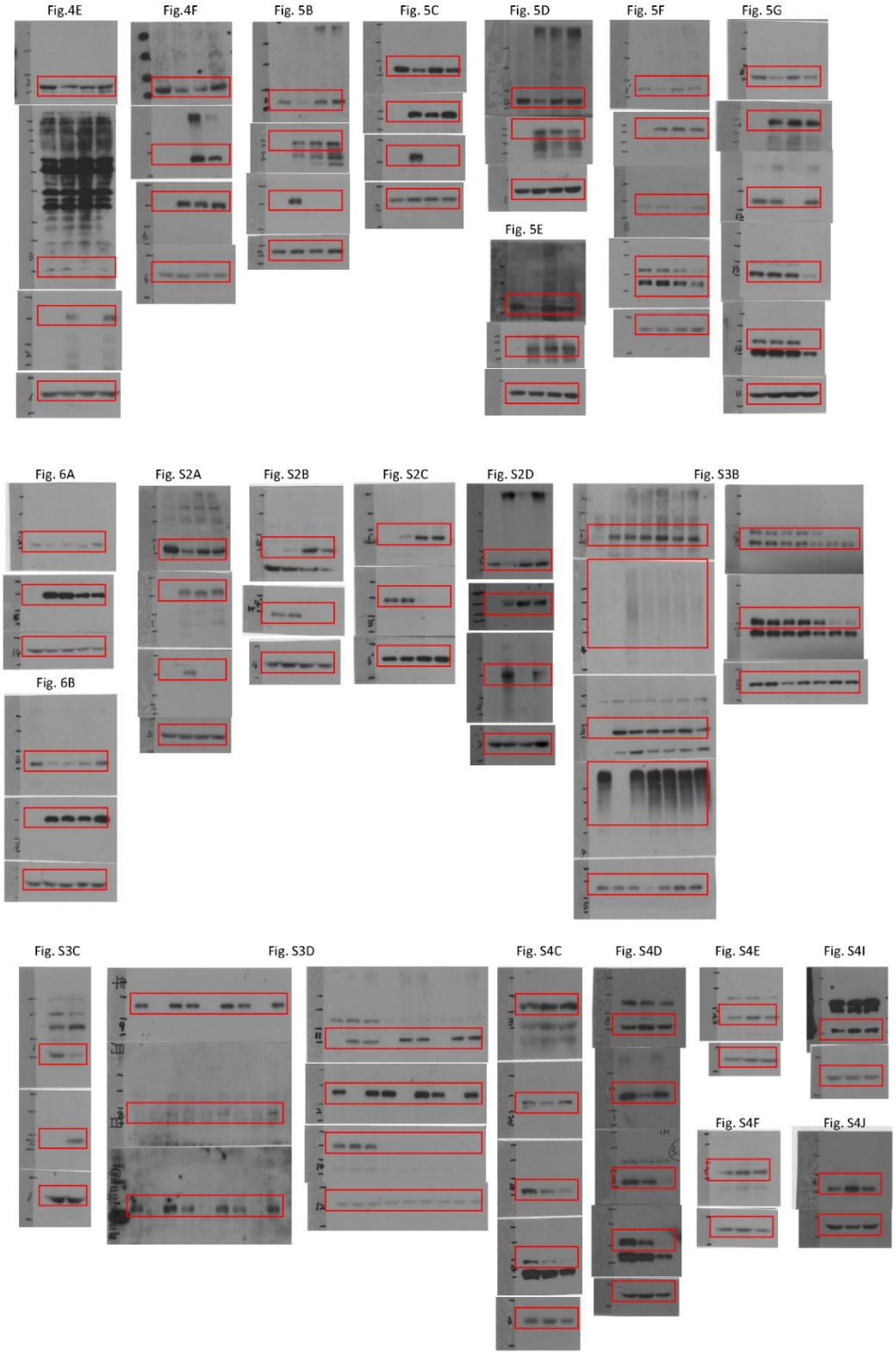

Fig. S6. Western blot–scanned films. Red boxes highlight lanes used in Fig. 4-6 and fig. S1-4