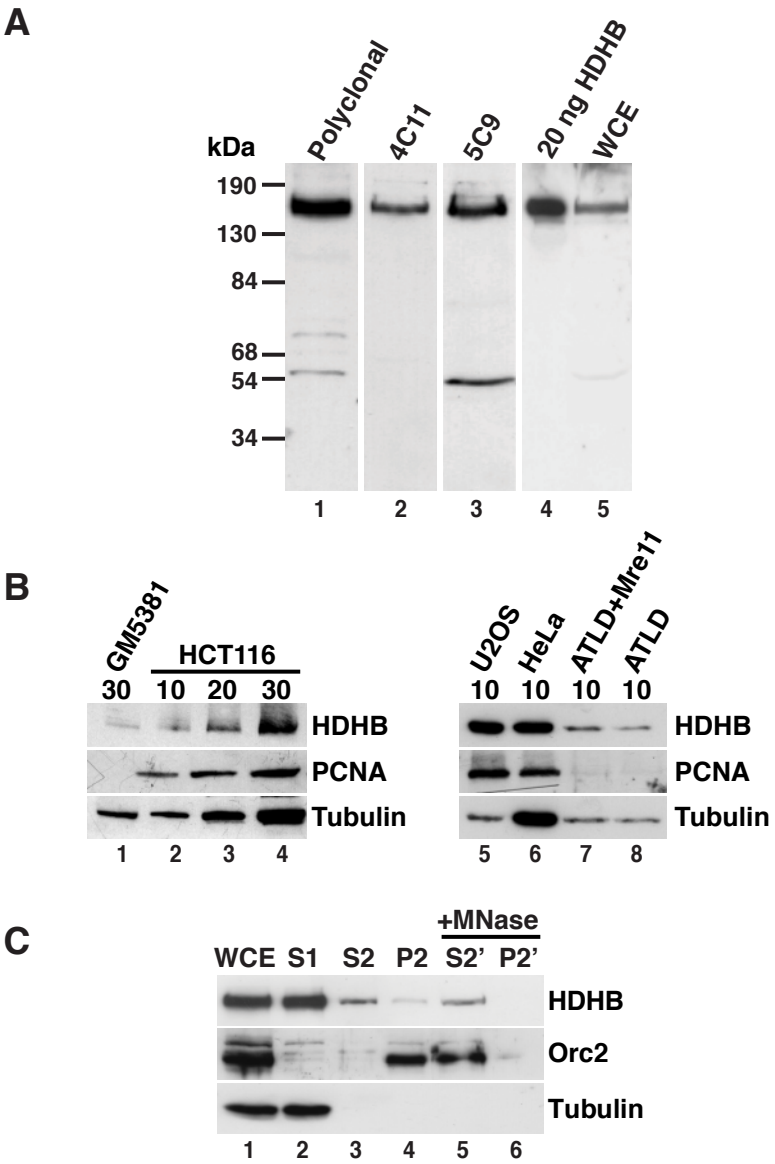

FIGURE S1. HDHB antibody specificity, HDHB expression levels and subcellular distribution.
A. U2OS whole cell extract (WCE) (lanes 1-3, 5) and purified HDHB (lane 4) were analyzed by SDS-PAGE and western blot using polyclonal rabbit (lanes 1, 4, 5) and monoclonal rat antibodies (lanes 2, 3).
B. Whole cell extracts (10-30 µg as indicated) from GM5381 (primary skin fibroblast) (lane 1), HCT116 (colorectal carcinoma cell line) (lanes 2-4), U2OS (osteosarcoma cell line) (lane 5), HeLa (cervical carcinoma cell line) (lane 6), ATLD+Mre11 (Mre11-complemented ATLD cells) (lane 7), ATLD (Mre11-deficient ataxia-telangiectasia-like disorder cells immortalized by hTERT expression) (lane 8) were separated by SDS-PAGE and analyzed by western blotting with antibodies against HDHB, tubulin, or PCNA, as indicated.
C. Whole cell extract (WCE), cytoplasmic (S1), nuclear soluble (S2), and chromatin (P2) fractions from asynchronously growing U2OS cells were prepared as described in Materials and Methods. In lanes 5, 6, nuclei were treated with micrococcal nuclease (MNase) before separating the nuclear soluble fraction from chromatin fraction. Proteins were visualized by western blotting with the antibodies indicated.

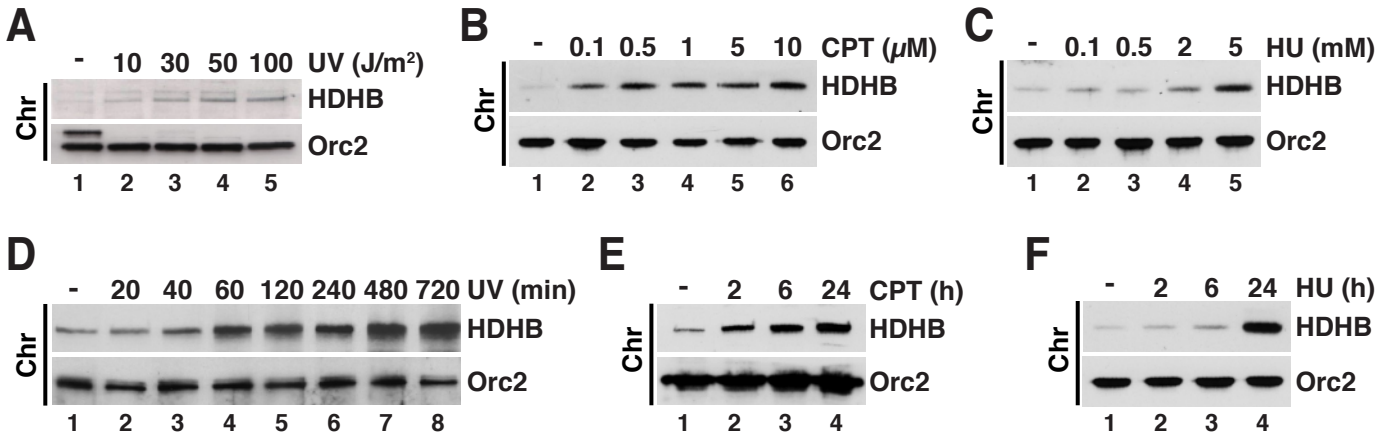

FIGURE S2. Damage-dependent accumulation of HDHB on chromatin.
A-C. Asynchronously growing U2OS cells were treated with indicated doses of UV (A), CPT (2 h) (B), or HU (2 h) (C) as indicated, then fractionated and analyzed as described in Fig. 1C.
D-F. Asynchronous cultures of U2OS cells were treated with 100 J/m^2 UV (D), 0.1 μM CPT (E), or 2 mM HU (F) for the indicated time periods, then fractionated and analyzed as described in Fig. 1C.

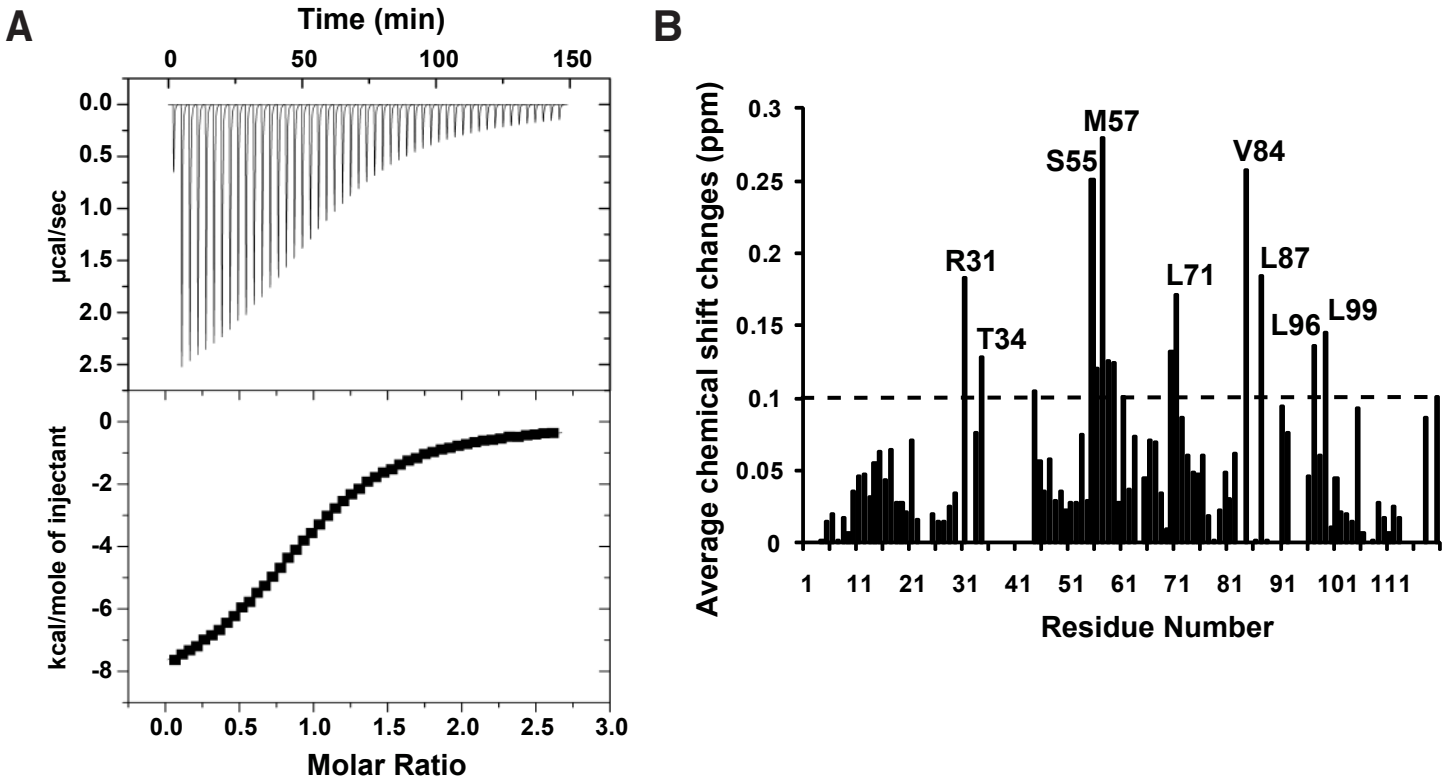

FIGURE S3. Analysis of the binding of HDHB peptide to RPA70N.
A. The binding isotherm of interaction between RPA70N and HDHB peptide showing the heat changes (upper) and the integrated heat changes that fit with a single site binding model (lower). K_d 15 +/- 0.05 µM.
B. The plot of the NMR chemical shift perturbations in RPA70N induced by the binding of HDHB (residues 493-517). The dashed line indicates one standard deviation above the mean. The absence of a bar for a given residue indicates that the peak for this residue was not assigned or that the peak disappeared upon the binding of the peptide.