Figure S1

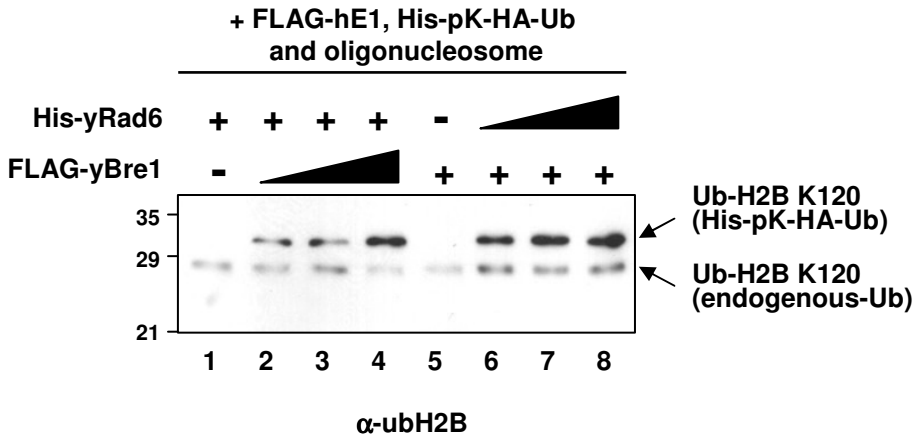

Fig. S1. Yeast Bre1 is limiting for H2B ubiquitylation. The chromatin ubiquitylation assay was performed with varying amounts of yBre1 and yRad6. Reactions contained 150 ng (lane 2), 300 ng (lane 3) or 600 ng (lanes 4-8) yBre1 and 50 ng (lane 6), 100 ng (lane 7) or 200 ng (lanes 1-4 and 8) yRad6.

Figure S2

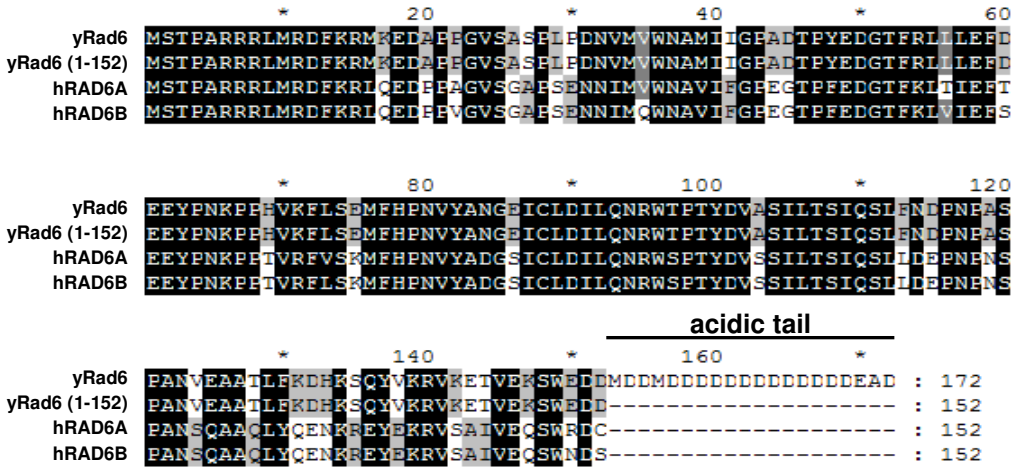

Fig. S2. Amino acid sequence alignment of yRad6, the yRad6 acidic tail deletion mutant (N152), hRAD6A and hRAD6B. The C-terminal acidic tail specific to yRad6 is indicated.

Figure S3

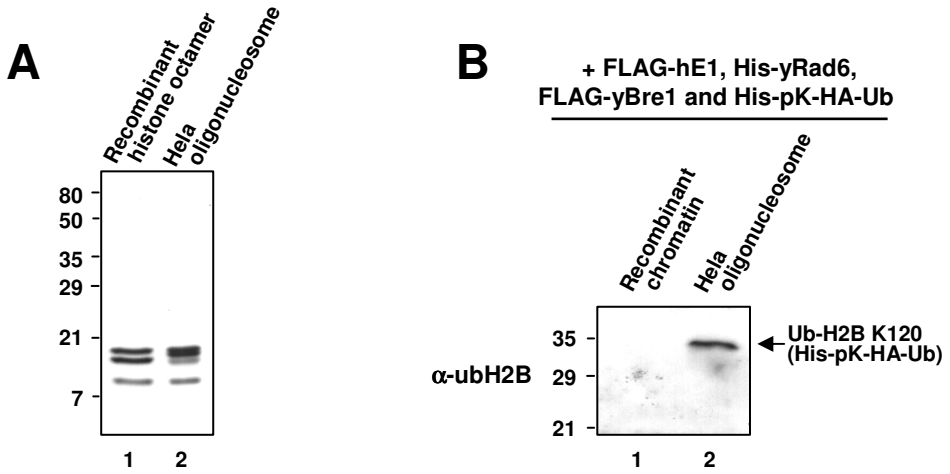

Fig. S3. Natural chromatins serve as preferred substrates for in vitro H2B ubiquitylation assay. *A.* Analyses of histones of recombinant octamer and natural oligonucleosome derived from HeLa cells by SDS-PAGE with Coomassie blue staining to ensure comparable levels of protein usage for in vitro chromatin ubiquitylation assay. *B.* Ubiquitylation substrate preference comparison of recombinant chromatin versus natural oligonucleosome. The chromatin ubiquitylation assay was performed as in Fig. 1*D* [except for inclusion of 350 ng (histone amount) chromatin] with indicated substrates for H2B ubiquitylation. Note that endogenous ubH2B is not detected in lane 2 because a much smaller amount (350 ng) of nucleosome substrate was used in this assay. Recombinant chromatin assembly employing recombinant histones, recombinant chromatin assembly factors and pML array DNA template was as descried [An et al., (2004) *Cell* **117**, 735-748].

Figure S4

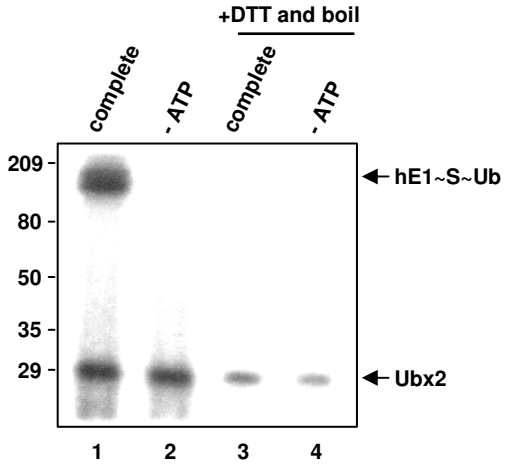

Fig. S4. Preparation of active recombinant hE1 ubiquitin activating enzyme. Purified hE1 was subjected to an ubiquitin thioester assay in the presence of ^{32}P-labeled ubiquitin. Thioester bond formation between E1 and ubiquitin was monitored by autoradiography. –ATP indicates the reaction without ATP. The ubiquitin thioester assay was performed as follows. Reactions containing 100 ng purified recombinant hE1, 25 mM HEPES-KOH [pH 7.5], 25 mM KCl, 5 mM $MgCl_2$, 2 mM ATP, 0.1 mM DTT and ^{32}P-labeled ubiquitin in 20 µl were incubated at 37 ℃ for 30 min and then stopped by the addition of 20 µl of loading buffer (100 mM Tris-Cl [pH 6.8], 4 % SDS, 20 % glycerol, 8 M urea). Half of each sample was supplemented with DTT to 50 mM and boiled for 3 min. Samples were subjected to SDS-PAGE and analyzed by autoradiography.