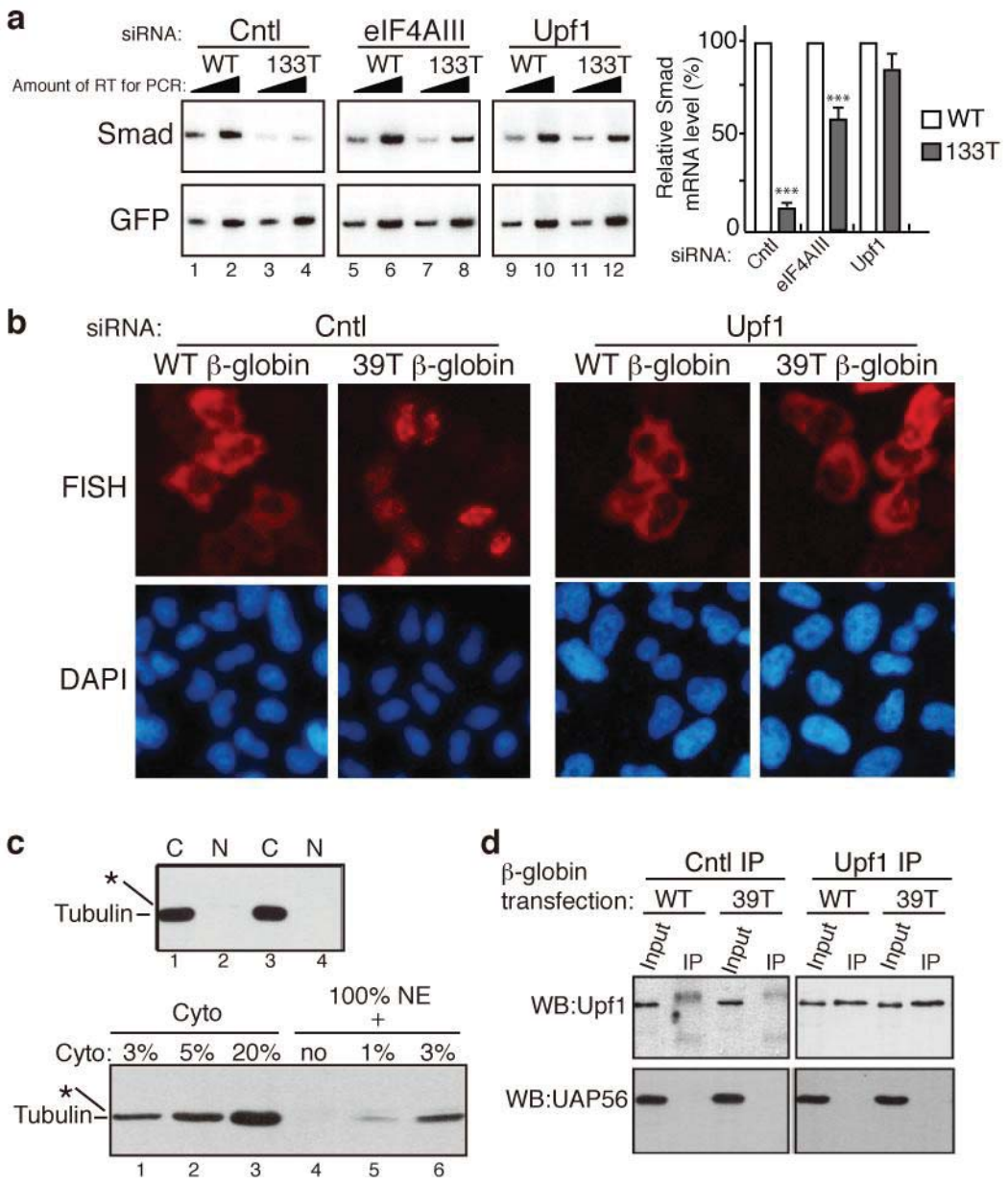

Figure S11. Knockdown of the NMD Factor Upf1 Inhibited the Nuclear Retention of PTC+ mRNAs, related to Figure 7. (a) eIF4AIII, Upf1, or non-targeting siRNAs were transiently transfected into HeLa cells, and 48 hrs post-transfection, WT or 133T Smad construct was co-transfected with GFP into these knockdown cells. 24 hrs after transfection, RNAs were extracted followed by RT-PCR using Smad and GFP primers. The ratio of mRNA level of Smad to GFP was quantified and indicated by the bars.

Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed as in Figure 1C. (b) Upf1 or non-targeting siRNA was transiently transfected into HeLa cells, and 48 hrs post-transfection, WT or 39T β-globin construct was transfected into these knockdown cells. 12 hrs after transfection, FISH was carried out. (c) HeLa cells were transfected with WT or 39T β-globin constructs, and 12 hrs later, nuclear and cytoplasmic extracts were prepared. Upper panel, equal volume of nuclear to cytoplasmic extract was separated by SDS-PAGE followed by western blot using an antibody to Tubulin or UAP56. Lower panel, the purity of nuclear fraction is shown. For comparison with the nuclear fraction, cytoplasmic fractions corresponding to 3, 5 and 20% (lanes 1-3) of the number of cells represented in the nuclear fraction (lane 4) were loaded, and the nuclear fraction was mixed with 1 or 3% cytoplasm (lanes 5 and 6). (d) Using an antibody to Upf1 or an unrelated protein, immunoprecipitations were carried out from nuclear extracts prepared from HeLa cells transfected with WT or 39T β-globin constructs. RNAs were extracted from immunoprecipitates followed by RT-PCR using primers to β-globin or Luciferase. The relative mRNA level of β-globin to Luciferase was quantified and indicated in the graph. Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed as in Figure 1C.