Supplementary data

Supplementary Materials and Methods

Figure legends

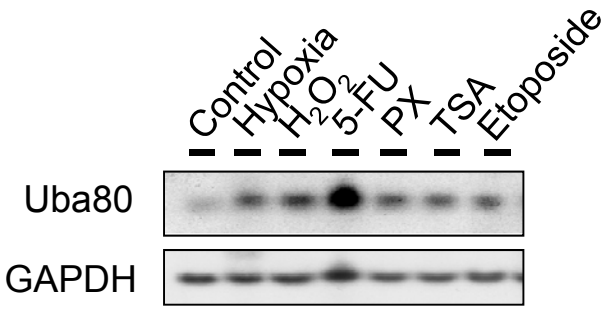
Supplementary Figure S1. Induction and Stability of *Uba80* mRNA. (**a**) *Uba80* mRNA induction by various genotoxic drugs. Hep3B cells were incubated under hypoxia, or treated for 48 h with H_2O_2 (0.5 mM), 5-FU (2 μg/ml), PX (100 nM), TSA (0.5 μM) or etoposide (50 μM) at their IC_{50} concentrations. Results shown for Northern blot analysis of *Uba80* and *GAPDH* gene expression are representative of at least three independent experiments. (**b**) Decay of the *RPS27a* transcript in Hep3B cells. Cells were treated with 4HPR alone, actinomycin D (ActD, 5 μg/ml) + 4HPR, or ActD + vehicle. Total RNA was extracted from the three sets and analyzed on Northern blots (left). The autoradiograms were analyzed using the LAS-3000 system and the *RPS27a* transcripts were normalized against the *18S* RNA levels (right).

Supplementary Figure S2. Cleavage and Specific Targeting of Ubiquitin and Ribosomal Protein. IF assay of Hep3B (**a**) and A549 cells (**b**) transiently transfected with 2 μg of plasmid encoding the wild-type Uba80 that was end-tagged with both GFP (N-terminal) and RFP (C-terminal), or with a vector control (pGR).

Supplementary Figure S3. Cleavage of the Ubiquitin Hybrid Proteins. (**a**) IF analysis in A549 cells transiently transfected with 2 μg of plasmid encoding the wild-type or the deletion mutant Uba52 that was end-tagged with both GFP (N-terminal) and RFP (C-terminal), or

with a vector control (pGR). ΔN4 and ΔN6 indicate the deletion mutants of Uba52 lacking four and six N-terminal amino acids, respectively (scale bar, 10 μm). (**b**) IF analysis in Hep3B cells transiently transfected with 2 μg of plasmid encoding the wild-type or mutant GFP-Uba52 or with a vector control (GFP). I77,I78 R indicates a mutant in which I77 and I78 are mutated to R. G76A/I77R indicates that G76 and I77 are mutated to A and R, respectively. G75,76A indicates that G75 and G76 are mutated to A. In I77R, I77 is mutated to R, and in G76A, G76 is mutated to A (scale bar, 10 μm). (**c**) Immunoblot analysis with GFP antibodies in Hep3B cells transiently transfected with 2 μg of plasmid encoding wild-type, the deletion mutants, and G75,76A mutants that were end-tagged, or with a vector control (pGR). Nuclear and cytoplasmic extracts were obtained by fractionation using NE-PER nuclear and cytoplasmic extraction reagents (Thermo Scientific, Rockford, IL) according to the manufacturer's protocol.

a



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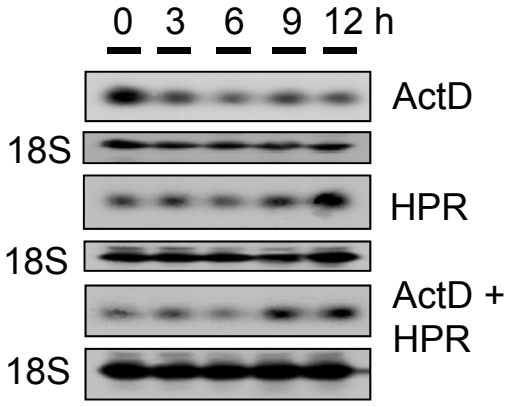
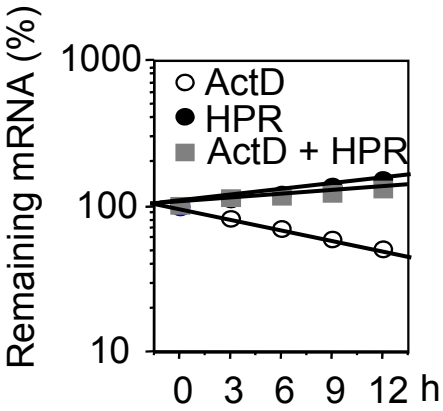
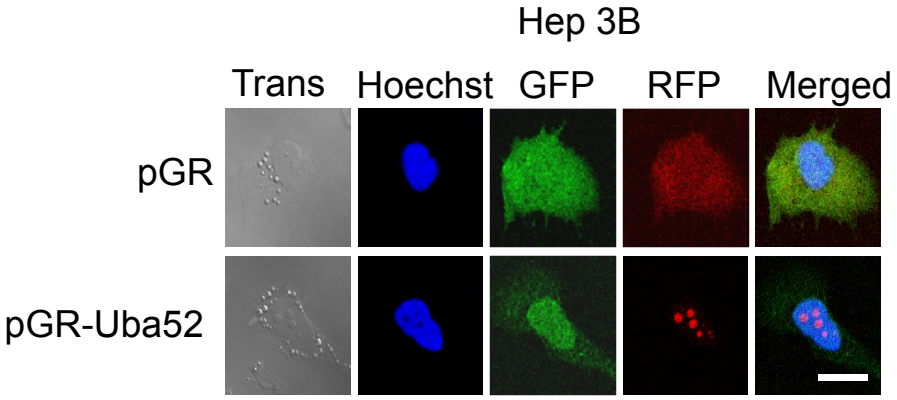




Supplementary Figure S1

a



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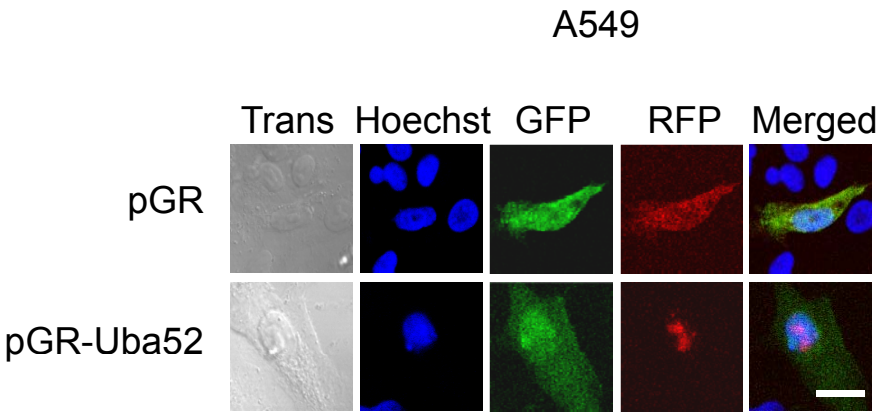
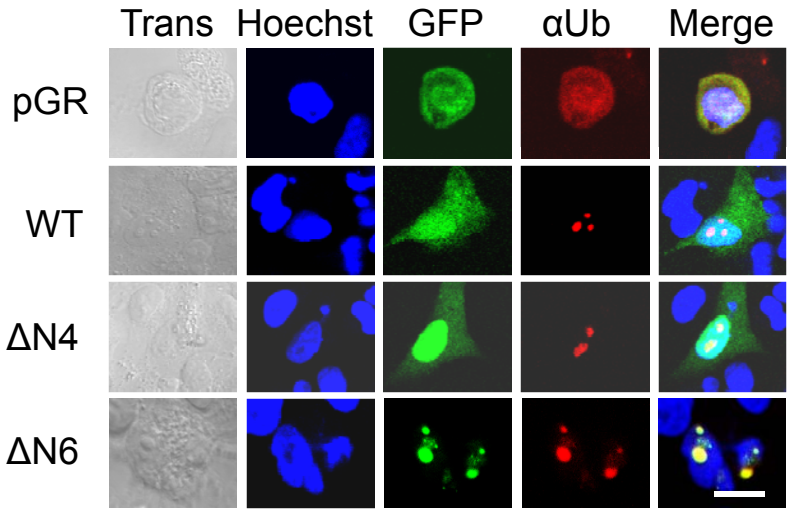


Supplementary Figure S2

a



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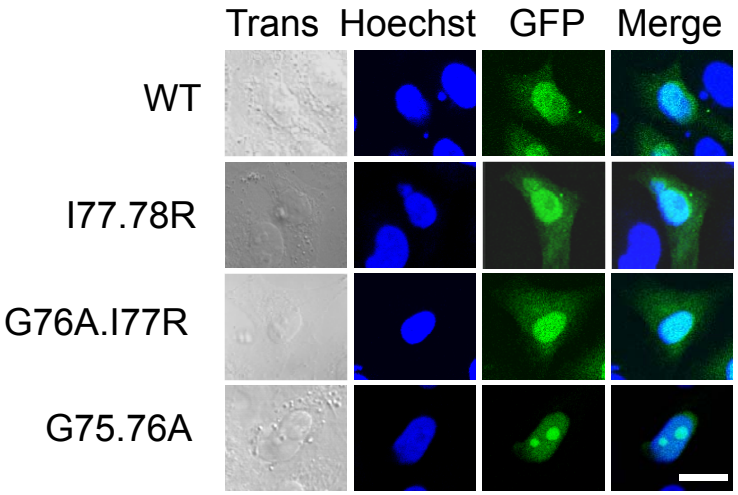

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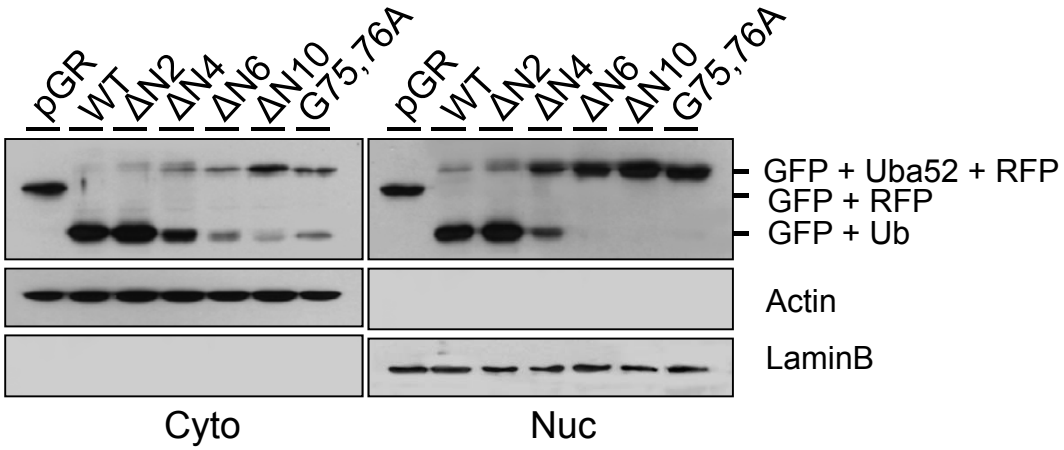

Supplementary Figure S3