SUPPLEMENTAL FIGURE LEGENDS

Figure S1: The UBN1 HRD is dispensable for HIRA binding. A 6His-UBN1(41-119)/HIRA(1-405) complex could be purified to homogeneity through multi-step chromatography, with the monodisperse size exclusion fractions shown.

Figure S2: UBN1 proteins with HRD truncations still form stable complexes with HIRA(1-472) **A**) GST-UBN1(41-119); **B**) GST-UBN1(41-109); **C**) GST-UBN1(41-89); and **D**) GST-UBN1(41-77). Left panels show the Ni-NTA purification, while the right panels show the GST purification of the same production. H–HIRA; U–UBN1; G–Proteolyzed GST moiety; Mw–molecular weight markers; In–Ni-NTA input; FT–NI-NTA flow-through; Elution–Ni-NTA fractions at increasing Imidazole concentration.

Figure S3. Full collection of the sedimentation equilibrium data for **A**) the HIRA(1-405)/UBN1(41-119) complex and **B**) the HIRA(1-405)/UBN1(41-175) complex. Data was collected at three centrifugation speeds (14,276, 22,277, and 32281 r.p.m.) and three protein concentrations (OD_{280} = 0.6, 0.4, and 0.2) and was globally fit to a $(UBN1)_1/(HIRA)_1$ single-species model. The data in A) fits to the molecular weight of the HIRA(1-405)/UBN1(41-119) complex (58.0 kDa) with an RMS deviation of 0.00950, while the data in B) fits to the molecular weight of the complex (67.0 kDa) with an RMS deviation of 0.00986.

Figure S4: 6His-UBN1(41-175)113LVPR114/HIRA(1-405) complex was purified to homogeneity and subjected to thrombin cleavage that cuts between UBN1 residues 113 and 114. Left lane: uncleaved complex after size exclusion chromatography; Right lane: Cleaved complex indicating the generation of the NHRD and HRD fragments by thrombin protease.

Figure S5. Ectopically expressed UBN1 WT, but not the (E61K,F62K) NHRD mutant, induces selected markers of cell senescence. Cells were infected with retroviruses as in Figure 8. **A**) 28 days later, cells were stained with DAPI and anti-p16 (Santa Cruz sc-56330: mouse monoclonal IgG_{2a}) and anti-cyclin A (Santa Cruz sc-751 rabbit polyclonal) antibodies and visualized by immunofluorescence. **B**) 28 days later cells were stained with DAPI and anti-HIRA (mouse monoclonal WC cocktail IgG1) and anti-PML (Santa Cruz sc-5621 rabbit polyclonal) antibodies and visualized by immunofluorescence.