Supplementary Figures

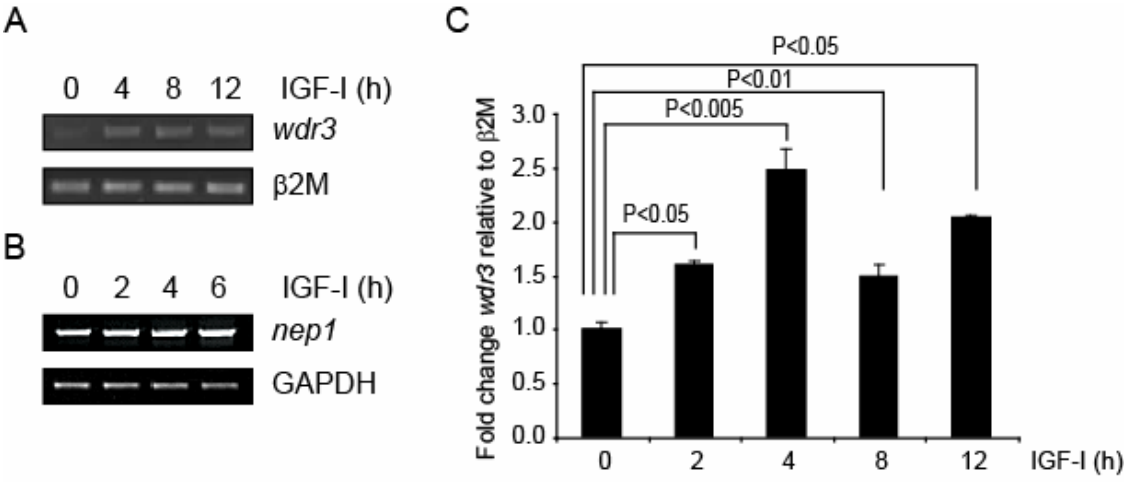

Figure S1. IGF-I induces expression of *wdr3* and *nep1* mRNA in R+ cells. *A*, R+ cells were serum starved for 4 h and stimulated with 100 ng/ml IGF-I for the indicated times. Total RNA was extracted and analysed by semi-quantitative RT-PCR using *wdr3* and β_2 microglobulin primers. Results represent one of three independent experiments with similar results. *B*, R+ cells were serum starved for 4 h and stimulated with 100 ng/ml IGF-I for the indicated times. Total RNA was extracted and analysed by semi-quantitative RT-PCR using *nep1* and GAPDH primers. Results represent one of three independent experiments with similar results. *C*, MCF-7 cells were serum starved for 12 h and stimulated with 10 ng/ml IGF-I for the indicated times. Total RNA was extracted and analysed by quantitative RT-PCR for expression of *wdr3* and β2M. The graph indicates fold change in *wdr3* mRNA expression relative to β2M, normalised to expression at time 0. Results are representative of three independent experiments. P values were obtained using Student's *t*-test.

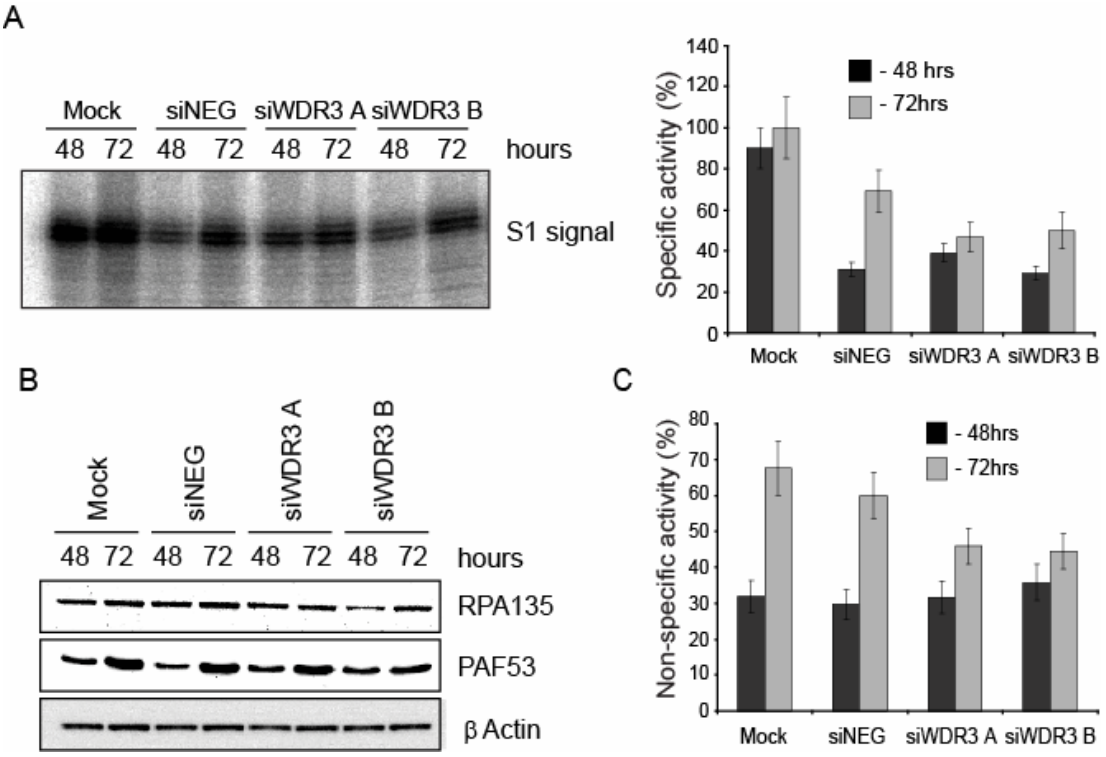

Figure S2. Suppression of WDR3 affects Pol I activity. *A,* Pol I specific activity in nuclear extracts from U2OS cells treated with Mock, control siRNA (siNEG), or siRNA targeting WDR3 (siWDR3 A and siWDR3 B) 24, 48, and 72 h post siRNA transfection was analysed by *in vitro* transcription assay. The same amount of protein was used in each reaction. Transcripts were analysed by S1 nuclease protection assay (left panel) and signals from triplicate samples were quantified using Fuji phosphor-imager and Aida software. The activity was expressed as a percentage from the highest signal (set 100%), (right panel). *B,* Nuclear extracts from U2OS cells treated with control siRNA or siRNA targeting WDR3 were analyzed for expression of RPA135, PAF53, and β-actin protein levels by western blot analysis 48 and 72 h post siRNA transfection. Results represent one of three independent experiments with similar results. *C,* Nuclear extracts from U2OS cells treated with control siRNA or siRNA targeting WDR3 were tested for non-specific transcription activity by *in vitro* transcription assay, signals from triplicate samples were quantified and non-specific activity was expressed as a percentage from the highest signal (set 100%).

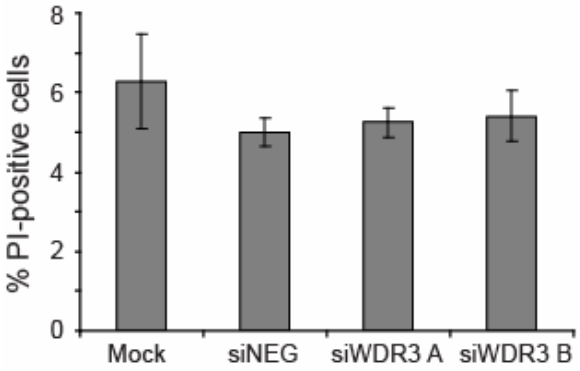

Figure S3. Suppression of WDR3 does not result in cell death. MCF-7 cells were Mock transfected, transfected with control siRNA, or transfected with siRNA targeting WDR3 and were assessed for cell death by quantification of propidium iodide-positive cells using flow cytometry. The graphs represent mean and standard deviation from three separate experiments.

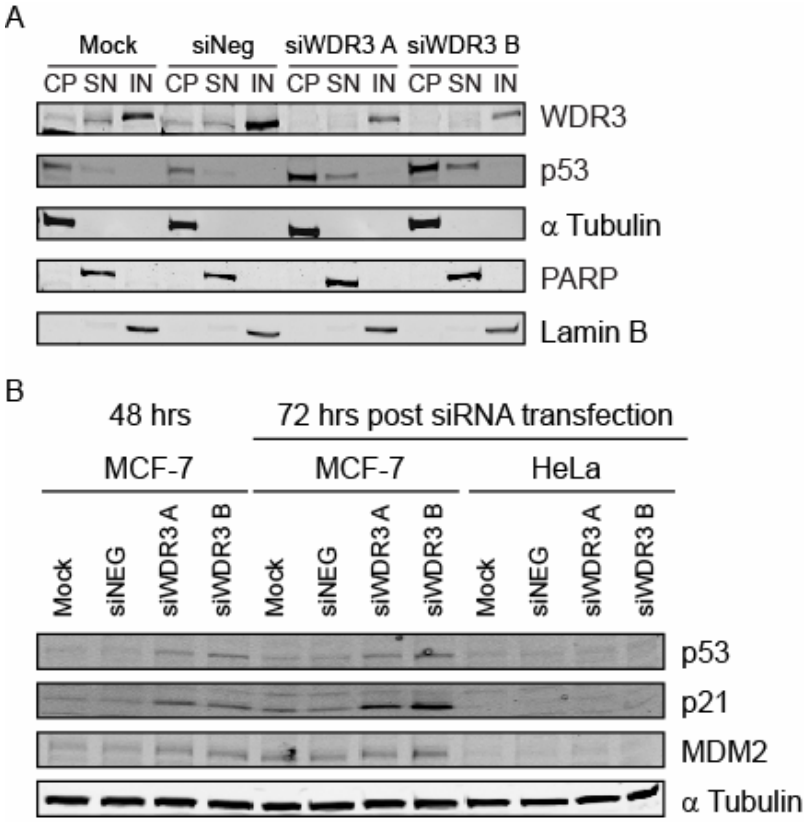

Figure S4. Suppression of WDR3 results in activation of the p53 stress response pathway in MCF-7 cells. *A,* MCF-7 cells were Mock-transfected, transfected with control siRNA, or siRNA targeting WDR3 for 72 h and fractionation into cytoplasmic (CP), soluble nuclear (SN), and insoluble nuclear (IN) fractions. Expression of WDR3, p53, α Tubulin as a marker for the cytoplasmic fraction, PARP as a marker for the soluble nuclear fraction, and Lamin B as a marker for the insoluble nuclear fraction were then analysed by western blot analysis. Results represent one of three independent experiments with similar results. *B,* MCF-7 and HeLa cells (Mock-transfected, control siRNA, and siRNA targeting WDR3) were cultured for the indicated time and analysed by western blotting for p53, p21, and MDM2 protein expression. α Tubulin is shown as a loading control. Results represent one of three independent experiments with similar results.

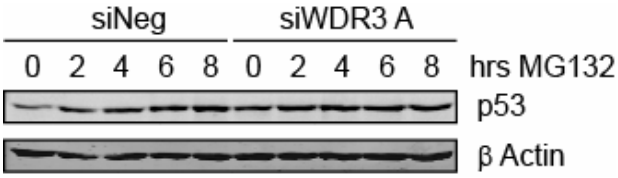

Figure S5. Suppression of WDR3 prevents the proteasomal degradation of p53. Following 48 h of culture MCF-7 cells (control siRNA-transfected cells (siNEG) and siWDR3 A) were treated with 10 μM MG132 for the indicated time. The expression of p53 and β Actin were then assessed by western blotting. Results represent one of three independent experiments with similar results.

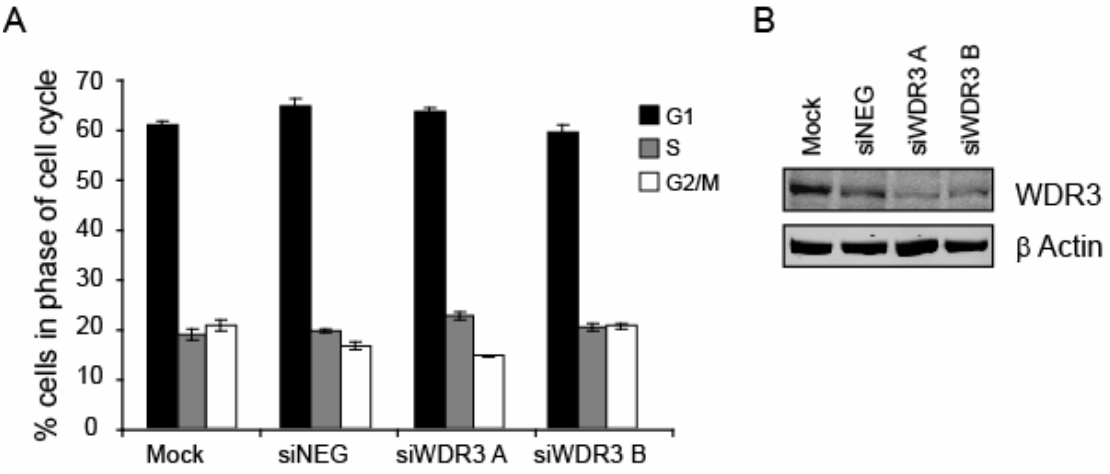

Figure S6. Cell cycle arrest due to suppression of WDR3 does not occur in the p53-deficient HeLa cell line. *A,* Cell cycle profiles of HeLa cells Mock transfected, transfected with control siRNA, or transfected with siRNA targeting WDR3 were stained with propidium iodide and DNA content was analysed by flow cytometry at 72 h post-transfection. The graph indicates the percentages of cells in G1 (black), S (grey), and G2/M (white) phases of the cell cycle from triplicate samples of three independent experiments. *B,* Levels of WDR3 protein expression in HeLa cells are shown with β Actin as a loading control. Results represent one of three independent experiments with similar results.

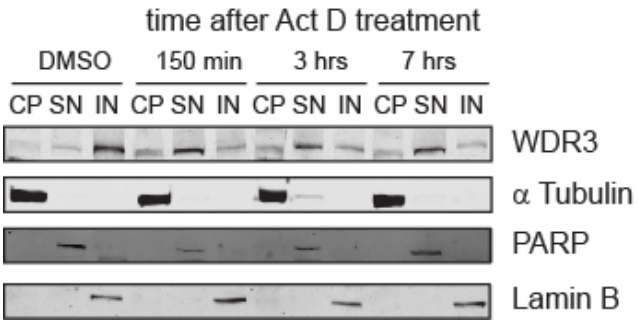

Figure S7. WDR3 protein is redistributed within the nucleus upon interference with ribosome biogenesis using low concentrations of Actinomycin D. MCF-7 cells were exposed to 40 ng/ml Actinomycin D for 1 h and cultured in complete media for the indicated times prior to subcellular fractionations into cytoplasmic (CP), soluble nuclear (SN), and insoluble nuclear (IN) fractions. Expression of WDR3, α Tubulin as a marker of the cytoplasmic fraction, PARP as a marker of the soluble nuclear fraction, and Lamin B as a marker of the insoluble nuclear fraction were analyzed by western blotting. Results represent one of three independent experiments with similar results.