Supplemental Materials

Molecular Biology of the Cell

Collier et al.

SUPPLEMENTAL MATERIALS

Translational control of a human *CDKN1A* mRNA splice variant regulates the fate of UVB-irradiated human keratinocytes

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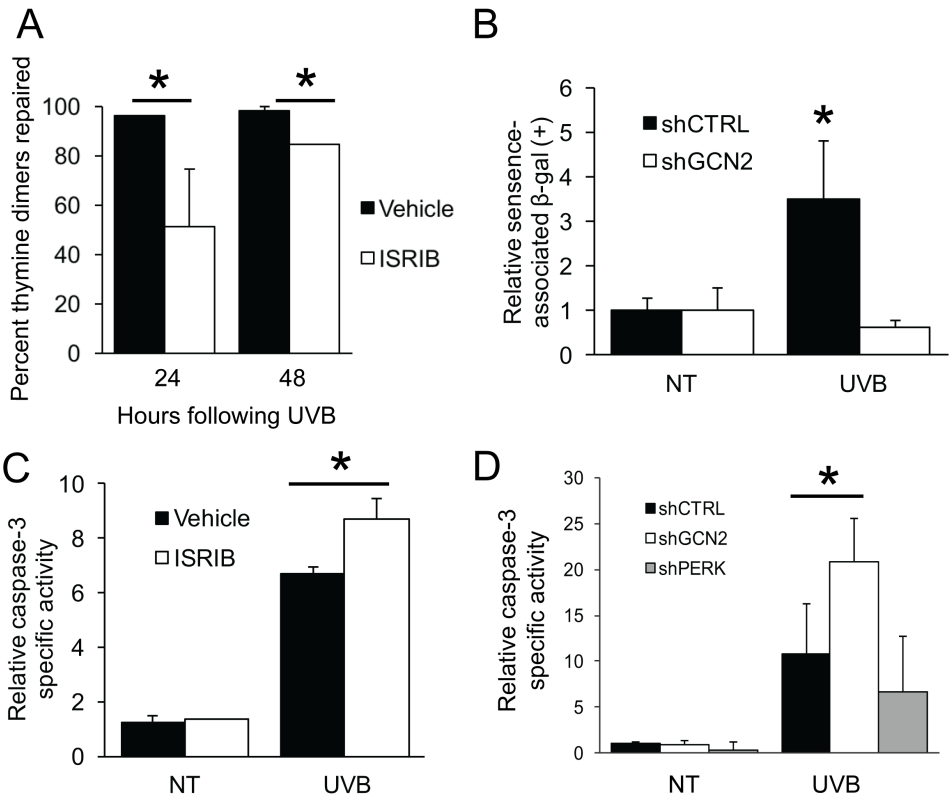
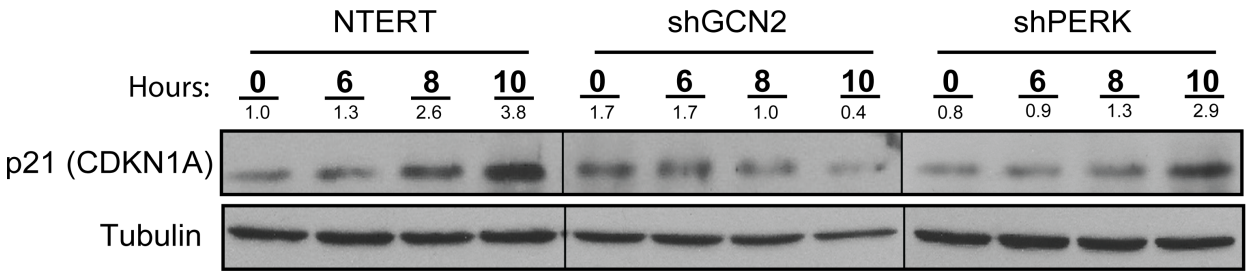
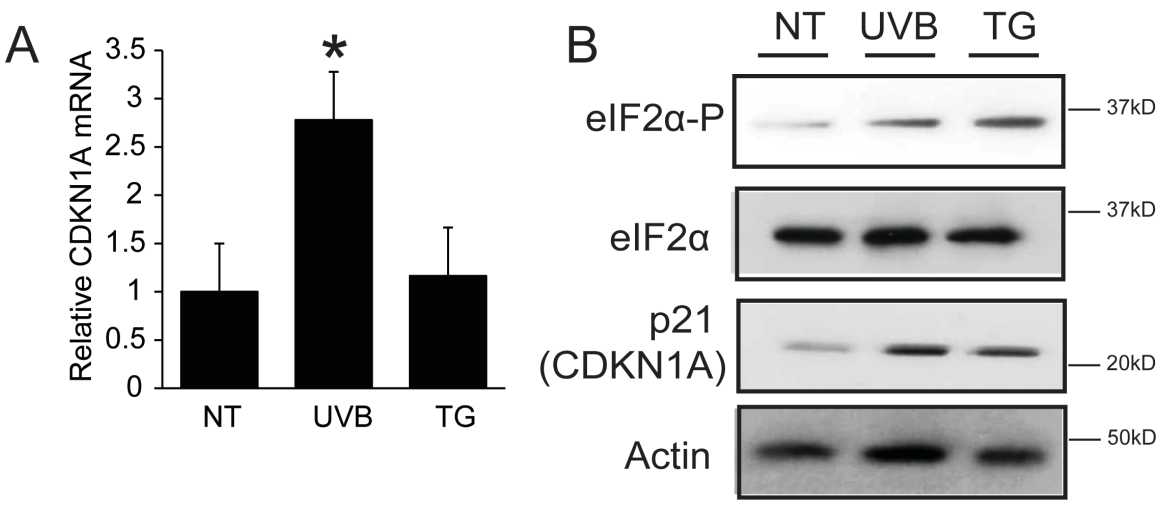


Supplemental Figure 1. Inhibition of the ISR affects DNA damage repair and cell fate. N-TERT cells were treated with vehicle or 200 nm ISRIB combined with 0 or 100 J/m^2 UVB. (A) Genomic DNA was isolated and immunoblot analysis was performed using antibody that measures thymine dimer content. (B) Control or shGCN2 N-TERTs were irradiated with 0 or 100 J/m^2 UVB and maintained in culture for 72 hours. Cells were then fixed and assayed for senescence-associated β-galactosidase activity. The ISR was inhibited in N-TERTs by either the addition of ISR (C) or GCN2 or PERK knockdown (D). Cells were subjected to 100 J/m^2 UVB (C) or 400 J/m^2 UVB (D), and capase-3 activity was determined.



Supplemental Figure 2. Knockdown of *GCN2* in N-TERT cells blocks induction of p21 expression following UVB exposure. N-TERT cells depleted for *GCN2* or *PERK* by shRNAs, or shRNA control (NTERT) were treated with 100 J/m^2 UVB and then cultured for the indicated number of hours. Alternatively, cells were not treated with UVB (0). Cells were collected, and p21 protein levels were measured in parallel experiments by SDS-PAGE and immunoblot analysis. Quantification of relative p21 protein is indicated above the immunoblot image.



Supplemental Figure 3. Thapsigargin regulation of *CDKN1A*. N-TERT cells were not treated (NT) or exposed to 1 uM TG or 100 J/m^2 UVB, as indicated. Lysates were collected following 6 hours of treatment and subjected to (A) qPCR to measure total *CDKN1A* mRNA levels or (B) immunoblot analyses to measure the levels of induced eIF2α-P and p21 protein expression. Sizes of protein markers are indicated to the right of the panels. * indicates $p<0.05$. Error bars represent mean +/- SD of three separate experiments.