Supplementary Materials

Supplementary Materials and Methods.
Human Subject Criteria. Inclusion criteria included: subjects must be over (or equal to) the age of 65 years old, must be able to understand the informed consent, and belong to Fitzpatrick skin types I or II (fair-skinned subjects). Subjects were excluded from the study if they: had a history of recent tanning bed use or UV treatments, had a history of photosensitive skin disease, were taking medications which were photosensitizing, had a history of diabetes, had a history of hypoglycemia, or had a history of abnormal scar formation. All portions of this study were conducted at the Indiana University Medical Center and approved by the Indiana University School of Medicine Institutional Review Board. Table S1 lists age and sex parameters for each of the human subject cohorts.

Quantitative Reverse-Transcriptase PCR. Homogenized tissue from dissected dermal sections was lysed using RNeasy kit (Qiagen) buffers. Cell lysates were then further homogenized using Shredder columns (Qiagen) and RNA isolation continued with RNeasy kit. All of the reagents used for RT and PCR are obtained from SuperArray Biosciences, Frederick, MD. The following were added to a 0.2 ml tube where first a genomic DNA elimination step was performed on 2 µg RNA total volume 10 µl was heated to 42°C for 5 minutes and chilled on ice. Next the reverse transcription cocktail was prepared and 10 µl added to the RNA; 2 µl RT enzyme mix, 4 µl RT buffer, 1 µl primer and external control mix, 3 µl RNase free water. Mixture was heated 42°C for 15 minutes, 95°C for 5 minutes and chilled on ice for experiments, the final volume of cDNA was 20 µl. qRT-PCR is performed using a LightCycler PCR (Roche Scientific, Fishers IN). Quantization of experimental qRT-PCR products was determined by comparison with external control qRT-PCR products from templates of a known copy number. Relative copy numbers of experimental mRNA are then determined following adjustment with actin controls from the same tissue.

Immunofluorescence. Paraffin-embedded sections were deparaffinized, hydrated, and rinsed with tris-buffered saline with tween 20 (TBS) (DAKO, Carpenteria, CA). Antigen
retrieval was performed using a water bath at 95°C for 20 minutes with DAKO Target Retrieval buffer. After cooling, the slides are rinsed with TBS, and the slides were then transferred to a clean 100 mm bacterial glass petri dish containing PBS-saturated filter paper under a strip of Parafilm. Primary antibodies (1:50) diluted in 3% BSA in TSB was added to the tissues. The lid was placed on the petri dish and the coverslips incubated for 1 hour at room temperature. The tissues were rinsed in PBS (three 10 minutes washes), and then the appropriate secondary antibody conjugated to the desired fluorochrome was added for 30 minutes at room temperature in the dark. The sections were washed as before, the edges blotted dry, and then mounted with coverslips using Fluoromount G. Antibodies used included: α-53BP1 (Abcam, Cambridge, MA), α-γH2AX (Millipore, Temecula, CA), α-Ki67 (NeoMarkers, Freemont, CA), and α-thymine dimers (Kamiya Biomedical, Seattle, WA).

Immunohistochemistry. Immunohistochemical detection of protein was be performed according to standard avidin-biotin technique (dilution 1:100) using heat induced epitope retrieval on the skin specimens. Slides were deparaffinized, hydrated, and rinsed with tris-buffered saline with tween 20 (TBS) (DAKO, Carpenteria, CA). Antigen retrieval was performed using a water bath at 95°C for 15 minutes with Vector Unmasking solution (in glycerol; 1:100). After cooling, the slides were rinsed with TBS, immersed in 3% H₂O₂ for 1 minute, blocked for 1 hour with Zymed CAS-Block, and incubated with primary antibody (anti-p53 antibody (recognizing both mutant and wild-type p53), clone DO-7, EMD Chemical, Gibbstown, New Jersey) overnight at 4°C. The slides were rinsed with TBS and incubated with the secondary antibody for 1 hour. After rinsing, the slides were incubated with Strep-Avidin HRP for 20 minutes, rinsed, and stain is developed with DAB for 3 minutes. The slides were rinsed and counterstained with hematoxylin.

UVB irradiation. UVB irradiation skin was accomplished using two Philips F20T12/UV sources as previously described [Lewis et al, 2010a]. The intensity of the UVB source
was measured prior to each use via an IL1700 radiometer and a SED240 UVB detector (International Light, Newburyport, MA) at a distance of 8 cm from the UVB source to the skin. All light emitted by the UVB source was blocked with the exception of the intended 1 x 1 cm area of the skin.

Young Adult Skin Versus Geriatric Skin Protocol. In accordance with the Declaration of Helsinki, written informed consent was obtained from all participants before enrollment in the study. All studies were approved by the Institutional Review Board at Indiana University Medical Center. Volunteers recruited for these experiments had Fitzpatrick Skin Types I or II (indicating fair-skinned individuals). Subjects were excluded from the study if they had a history of recent tanning bed use or therapeutic UV treatments, had a history of photosensitive skin disease, were taking medications which are photosensitizing, had allergies to xylocaine anesthesia, or had a history of abnormal scar formation. Subjects who met these criteria were separated into two groups; the first group consisted of individuals between the ages of 20 and 28 years old while the second group consisted of individuals greater than 65 years old. On Day 1 of the protocol, a 2 cm² area of the subject's lower back was isolated and irradiated with dose of 350 J/m² of UVB. In Fitzpatrick Skin Types I and II, this dose of UVB is sufficient to cause a minimal erythematous reaction. Twenty-four hours following UVB exposure, the irradiated skin, as well as unirradiated adjacent skin, were removed by punch biopsy, (a 4 mm punch biopsy of the UVB-treated area and a 3 mm punch biopsy of uninvolved skin on the same hip at least 10 cm away). Thin paraffin-embedded sections from unirradiated and UVB-irradiated biopsies were simultaneously stained with antibodies to Ki67 and thymidine dimers. Secondary antibodies that specifically detect only one of the primary antibodies are conjugated to the fluorescent dyes AlexaFluor 488 (detecting Ki67, emitting green wavelengths), and AlexaFluor 568 (detecting thymidine dimers, emitting red wavelengths). Images were captured sequentially along the entire length of the biopsy specimen (3mm non-irradiated, 4mm irradiated) using a Nikon Optiphot fluorescent microscope. These images were analyzed by counting the number of keratinocytes in contact with the basement membrane that are Ki67(+), thymidine dimer(+), and Ki67(+):thymidine dimer(+). These
numbers were expressed as a percentage of total basal layer keratinocytes in the biopsy specimen (determined by counting basal layer keratinocytes for each specimen on H&E-stained slides).

Dermabrasion of Geriatric Skin. Prior to treatment, a region of sun-protected hip/buttock skin was photographed. Next, an approximately 5 x 5 cm area of the subject's lower hip/buttock skin was isolated and anesthetized with xylocaine anesthesia. Under sterile conditions the localized area of skin was then abraded with sterile, coarse (#60) sandpaper down to the mid dermis, with complete removal of all epidermis and superficial dermis. The wounded area was bandaged with moist, occlusive dressings and the volunteer was instructed to change the dressing twice daily until the wound is re-epithelized in 1-2 weeks. Approximately three months later (~Day 90 +/- 7 days) the volunteer returned to the clinic and a localized area 1 x 1 cm of either dermabrasion or untreated normal skin on the opposite hip/buttock was irradiated with dose of 350 J/m² of UVB. In Fitzpatrick Skin Types I and II, this dose of UVB is sufficient to cause a minimal erythematous reaction. Permanent marker was used to outline the areas of skin that was irradiated. Twenty-four hours following UVB exposure, photographs were taken of the skin to document the extent of the UVB reaction. The irradiated skin, as well as unirradiated adjacent skin, was removed by punch biopsy, (4 mm punch biopsies of the UVB-treated skin and 3 mm punch biopsies of unirradiated skin; 4 biopsies per individual).
Figure S1. Comparison of geriatric markers and the UVB response in sun-protected skin and chronically sun-exposed skin in geriatric patients. (A) The percentage of senescent fibroblasts in the papillary dermis of biopsies from the upper buttocks (sun-protected) or dorsal forearm (sun-exposed) skin from geriatric (>65 years old) volunteers was determined using the methodology described in Fig 1. No significant difference was observed between the two cohorts (p=0.45, student t-test; n=16 (sun-protected) and n=6 (sun-exposed)). (B) The relative expression of the IGF-1 gene was determined by QRT-PCR analysis on the cohorts described in Fig. S1. Although the sun-exposed cohort appears to have a slightly higher level of IGF-1 expression than the sun-protected cohort, the difference was not statistically significant (p=0.16, student t-test; n=28 (sun-protected) and n=26 (sun-exposed)). (C) The extent of UVB-induced DNA damage in geriatric sun-exposed or sun-protected skin was determined by quantifying the number of basal layer keratinocytes containing thymine dimer lesions at twenty-four hours following an approximately one MED dose of UVB. No significant difference was observed between the two cohorts (p=0.15, student t-test; n=22 (sun-protected) and n=9 (sun-exposed)). (D) The UVB response of geriatric sun-exposed and sun-protected skin was determined as previously described [17-18] by determining the number of keratinocytes containing UVB-damaged DNA (TD+) and markers of cell proliferation (Ki67+) twenty-four hours following UVB exposure. While the sun-exposed skin trended to have fewer double-positive keratinocytes, there was no significant difference between the two cohorts (p=0.16, student t-test; n=24 (sun-protected) and n=8 (sun-exposed)).
Changes in the physical dimensions of the dermis and epidermis of geriatric skin following FLR treatment were evaluated on both sun-protected and sun-exposed skin using Nikon Elements image analysis software [18] which determines the proportion of the 3mm biopsy (expressed as an ‘area’) that encompasses the epidermis and the papillary dermis. The length of the biopsy contained on the stained section of the skin was also determined so that the area of each component of the skin can be normalized and compared. Error bars indicate SEM; asterisks identify significant difference from control values (p<0.01; two-tailed student t-test). 

(B) The density of fibroblasts in the papillary dermis was quantified in untreated and FLR-treated geriatric skin. Error bars indicate SEM; asterisks identify significant difference from control values (p<0.01; two-tailed student t-test).
Figure S3. Prophylactic FLR-treatment reduces the number of UVB-induced DNA-damaged keratinocytes in geriatric skin. Geriatric skin (both sun-protected and sun-exposed) were treated with FLR therapy as described in the Methods. At three months post-treatment, a small section of treated skin was irradiated with a dose of 350 J/m² of UVB. Twenty-four hours after UVB exposure, the irradiated skin was removed by biopsy and the number of basal layer keratinocytes containing UVB-induced thymine dimers was determined as described in the Methods. Error bars indicate SEM; asterisk denotes statistical significance of Geriatric Control values from all other cohorts (p<0.05; individual paired t-test). Young adult (n=6); geriatric control (n=22); geriatric DA (n=6); geriatric FLR sun-protected (n=8); geriatric sun-exposed control (n=8); geriatric FLR sun-exposed (n=8). NOTE: Young adult and geriatric DA data are presented in this figure for comparison; similar data have been previously reported in another format [Lewis et al, 2010a; Lewis et al, 2011].
### Table SI. Age and gender of patient cohorts

| Patient Parameters | YA<sup>a</sup> | GA control<sup>b</sup> | GA DA<sup>c</sup> | GA FLR sun-protected<sup>d</sup> | GA FLR sun-exposed<sup>e</sup> |
|--------------------|----------------|-------------------------|------------------|---------------------------------|-------------------------------|
| Age range (years)  | 22-28          | 66-89                   | 67-85            | 68-89                           | 66-80                         |
| Average age (years)| 25             | 78                      | 78               | 82                              | 75                            |
| % male             | 67             | 82                      | 86               | 71                              | 100                           |
| # of subjects      | 6              | 33                      | 7                | 7                               | 8                             |

<sup>a</sup> young adult  
<sup>b</sup> geriatric adult, controls  
<sup>c</sup> geriatric adult, dermabrasion  
<sup>d</sup> geriatric adult, fractionated laser resurfacing, sun-protected skin  
<sup>e</sup> geriatric adult, fractionated laser resurfacing, sun-exposed skin