Ponugoti et al., http://www.jcb.org/cgi/content/full/jcb.201305074/DC1

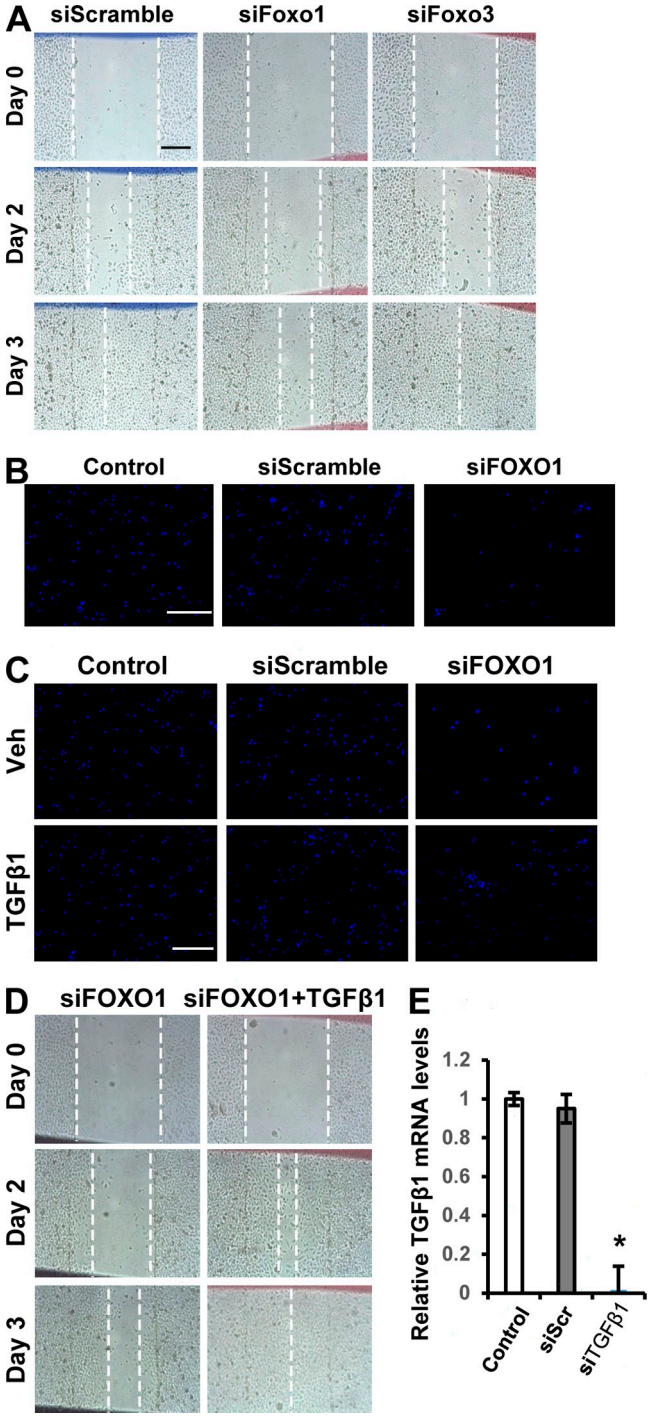

Figure S1. **Effect of FOXO1/FOXO3 knockdown on keratinocyte migration in the absence and presence of TGF- β1.** (A) Photomicrographs of scratch closure at the indicated time points in NHEK control cultures or FOXO1/FOXO3 knockdown cells after scratch wounding. Bar, 500 µm. (B and C) Cell migration was assessed by transwell migration assay; the cells that migrated across the transwells were stained with DAPI and the migrated cells were photographed. Bar, 200 µm. (D) Photomicrographs of scratch closure at the indicated time points in FOXO1 knockdown cells without or with TGF-β1 after scratch wounding. Bar, 500 µm. (E) NHEK cells were transfected with control or TGF-β1 siRNAs for 48 h. TGF-β1 levels were analyzed by real-time PCR. *, $P < 0.05$ vs. scrambled siRNA.

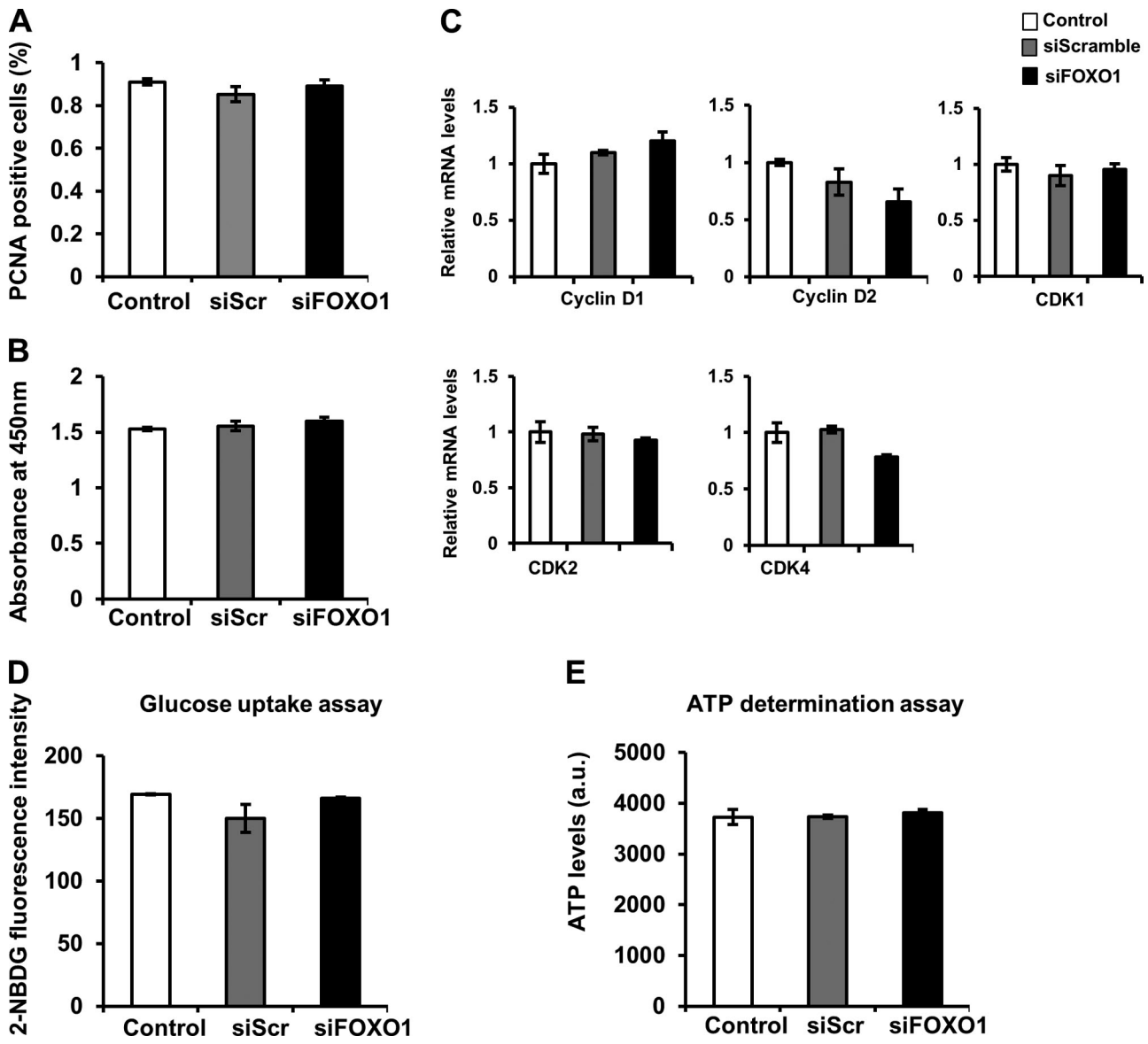

Figure S2. **Effect of FOXO1 knockdown on proliferation, glucose uptake, or ATP production in keratinocytes.** NHEK cells were transfected with control or FOXO1 siRNAs for 48 h. (A) Proliferative keratinocytes were determined by PCNA immunofluorescence or (B) BrdU ELISA. BrdU incorporation was determined by measuring absorbance at 450 nm. (C) Real-time PCR analysis of cyclin D1, cyclin D2, CDK1, CDK2, and CDK4 mRNA levels in FOXO1 knockdown keratinocytes. (D) Cells were incubated with 2-NBDG and the amount of NBDG uptake was measured by flow cytometry. (E) Intracellular ATP levels were measured using ATP determination kit. a.u., arbitrary units. Data show mean ± SEM of at least three independent measurements.

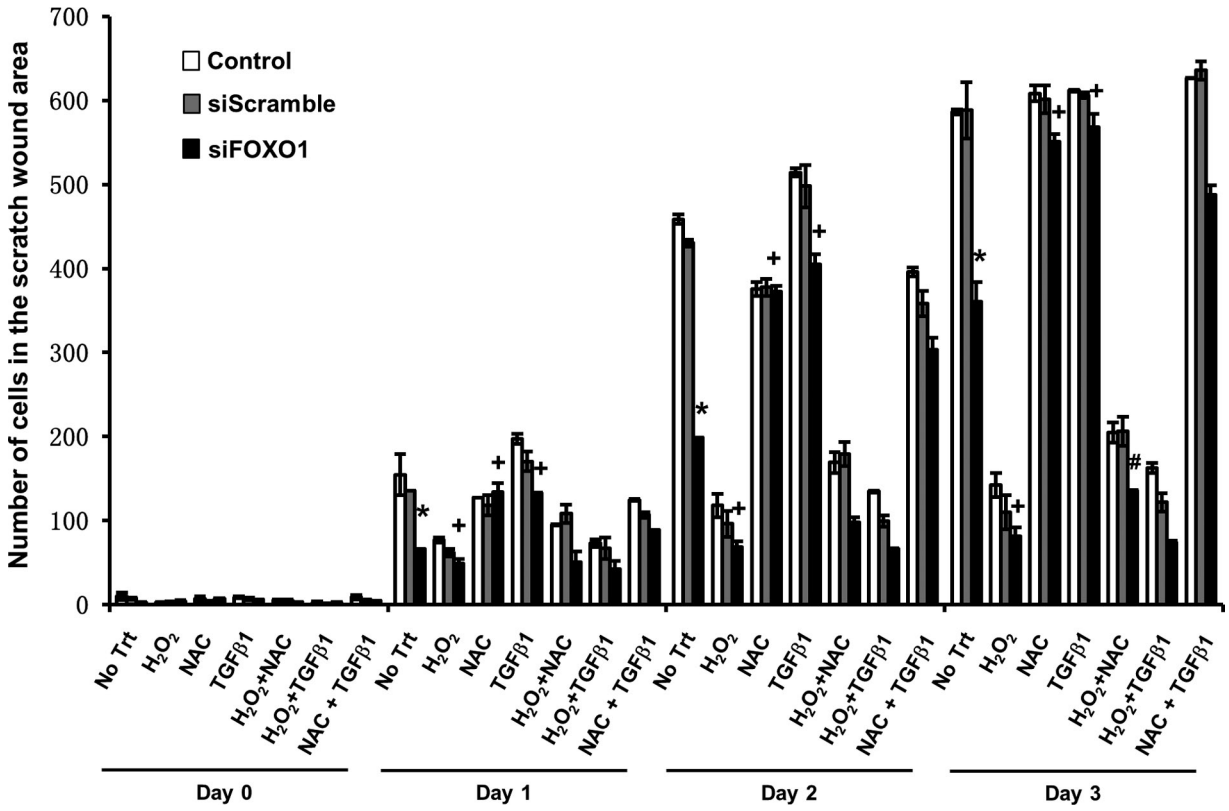

Figure S3. **Increased oxidative stress impairs keratinocyte migration of in vitro scratch wound.** NHEK cells were transfected with control or FOXO1 siRNA for 48 h and then treated with 150 μm H_2O_2, 1 mM NAC, 2 ng/ml TGF-β1, or various combinations of these agents after scratching. The number of cells in the scratched area was counted. Data show mean ± SEM of at least three independent measurements. *, $P < 0.05$ vs. scrambled siRNA; $^{+}$, $P < 0.05$ vs. siFOXO1; $^{\#}$, $P < 0.05$ vs. H_2O_2-treated siFOXO1.

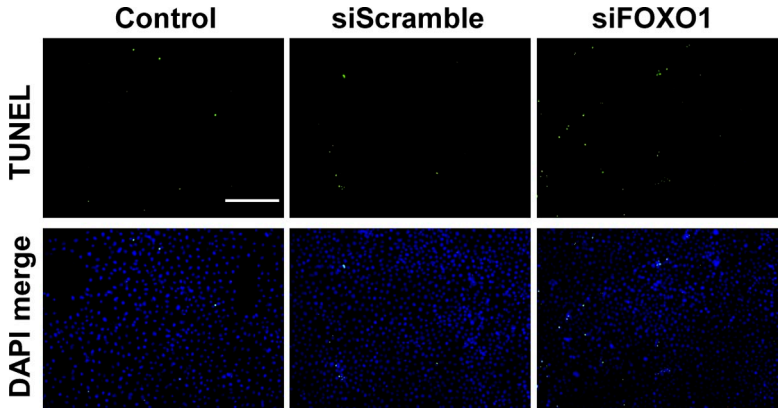

Figure S4. **FOXO1 knockdown results in increased keratinocyte apoptosis.** NHEK cells were transfected with control or FOXO1 siRNAs for 48 h. Apoptotic keratinocytes were measured by TUNEL staining. Green fluorescence indicates TUNEL-positive cells. Blue fluorescence indicates DAPI nuclear staining. Bar, 200 μm.

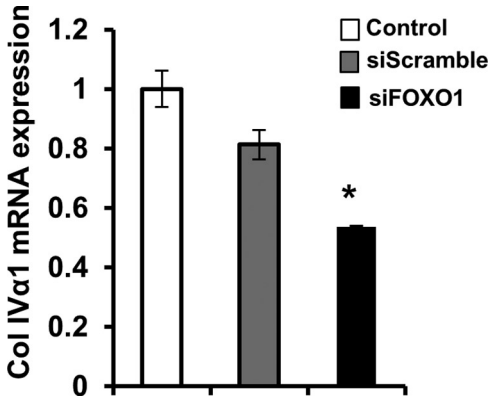

Figure S5. **Silencing FOXO1 in keratinocytes results in decreased collagen IVα1 mRNA levels.** NHEK cells were transfected with control or FOXO1 siRNAs for 48 h. (A) Real-time PCR analysis of collagen IVα1 mRNA levels in FOXO1 knockdown keratinocytes. *, $P < 0.05$ vs. scrambled siRNA.