



Fig. S1. Variable SA-β-gal staining in freshly isolated dermal cells

Each box shows SA-β-gal staining in freshly isolated dermal cells from each individual donor. The designation of each sample is shown on the top. The number following the letter “Y” is the age of the donor. Scale bar = 100 μm.

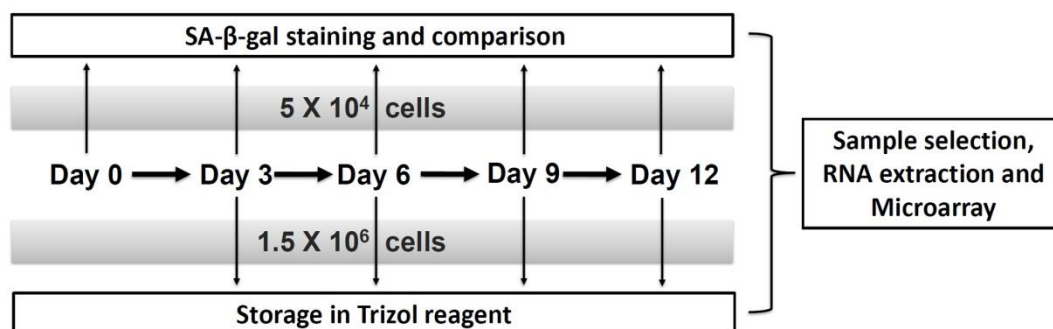


Fig. S2. The sampling protocol for microarray experiments

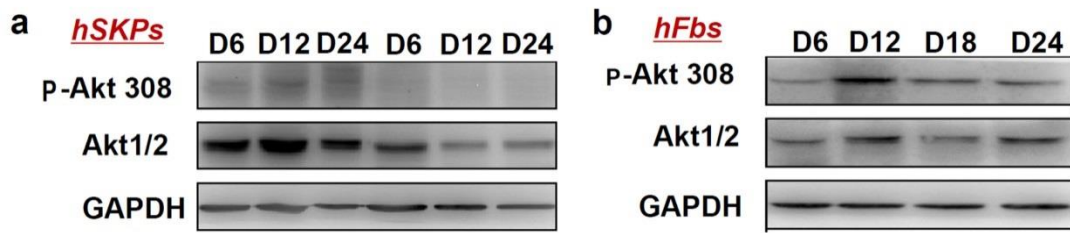


Fig. S3. Different levels of Akt phosphorylation in hSKPs and hFbs

Western blots showing the expression of p-Akt 308, Akt1/2 and GAPDH proteins in hSKPs (a) and hFbs (b) on different days of culture.

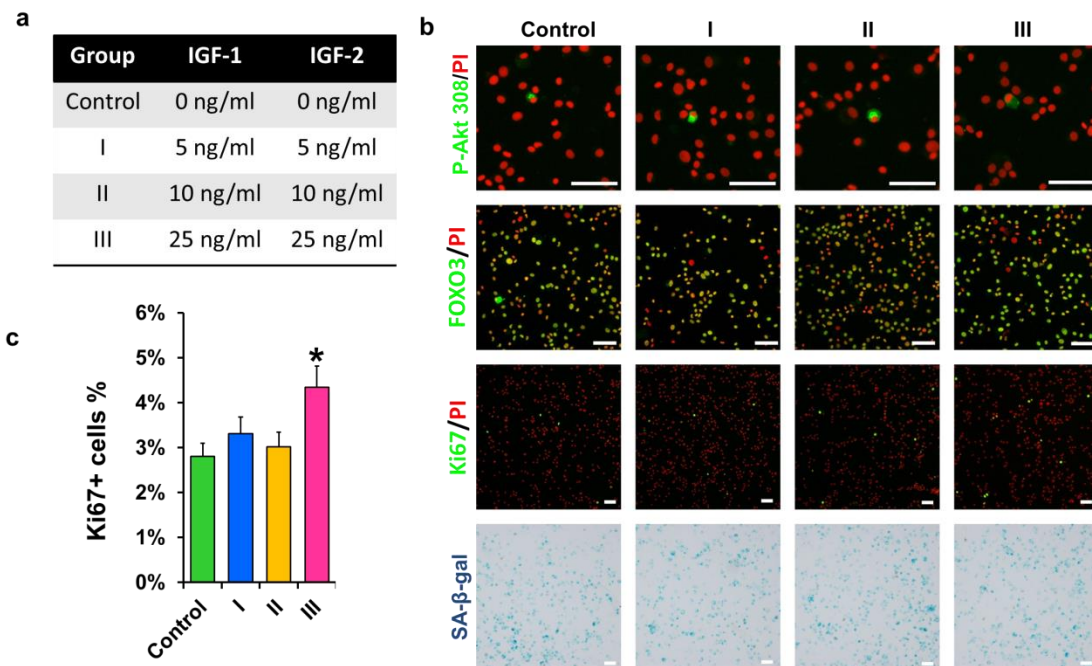
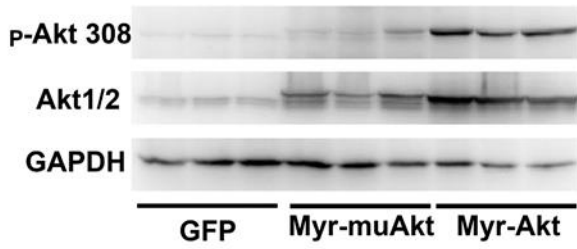


Fig. S4. Effect of IGFs in hSKPs

(a) Concentrations of IGF1 and IGF2 in different groups. (b) Phospho-Akt Thr308, FOXO3, Ki67 and SA-β-gal staining in hSKPs treated with different concentrations of IGF1 and IGF2. (c) Statistical analysis of Ki67+ cell ratio in hSKPs treated with different concentrations of IGF1 and IGF2. Scale bar = 50 μm



GFP and Myr-muAkt are used as controls.

Fig. S5. Western blots showing Akt phosphorylation in hSKPs after lentiviral transduction

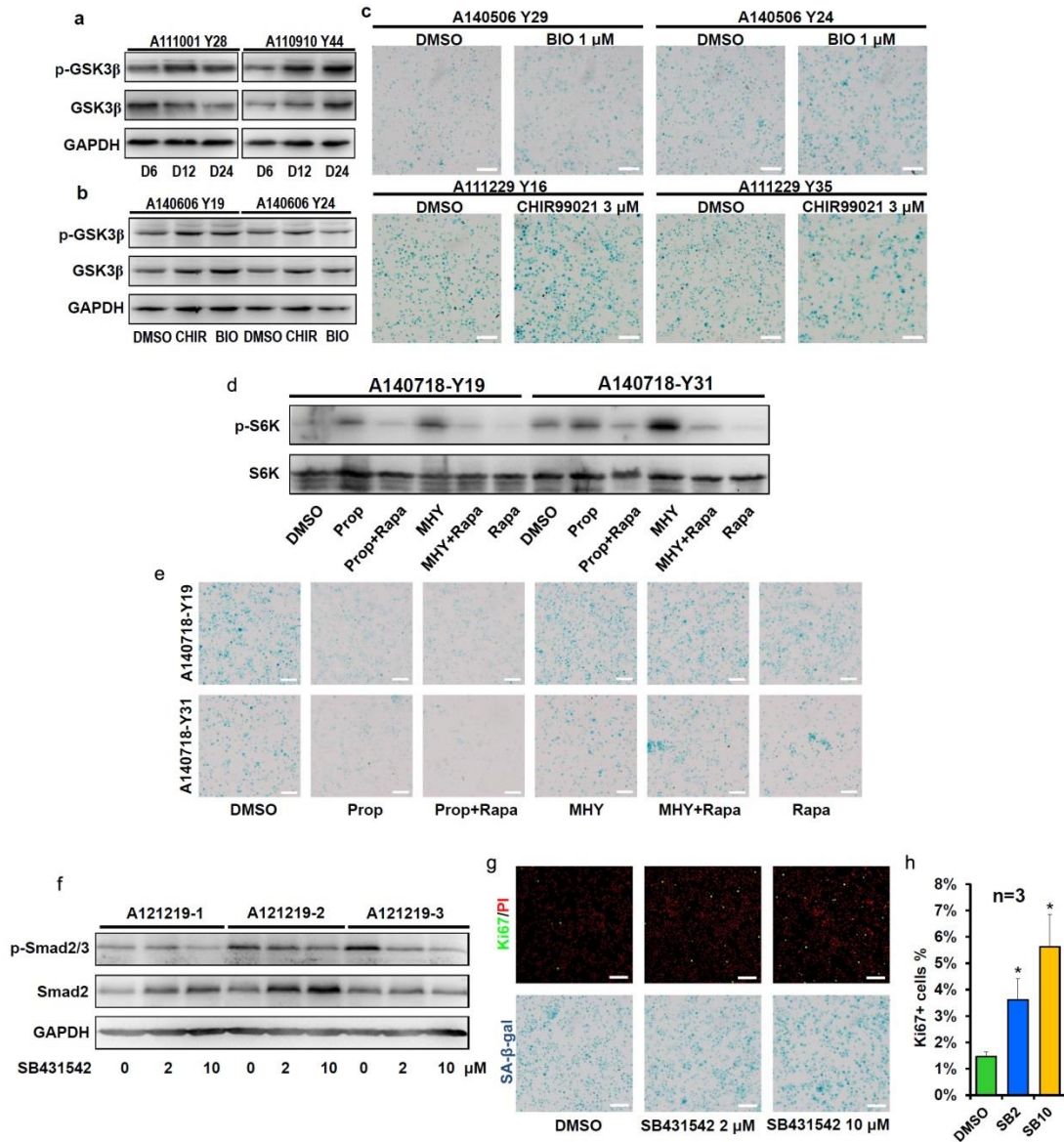


Fig. S6. GSK3β, mTOR and TGF-β in hSKP senescence

(a) Western blots showing the expression of phospho-GSK3 β , and GAPDH proteins in two hSKP samples on different days of culture. (b) Western blots showing the expression of p-GSK3 β , GSK3 β and GAPDH proteins in two hSKP samples treated with chemical GSK3 β inhibitors or vehicle DMSO. CHIR is short for CHIR99021. (c) SA- β -gal staining in hSKPs treated with chemical GSK3 β inhibitors or vehicle DMSO. Each experiment was repeated with two independent samples. (d) Western blots showing the expression of p-S6 and S6 ribosomal proteins in two hSKP samples treated with different combinations of mTOR activators and inhibitor Rapamycin as well as vehicle DMSO. (e) SA- β -gal staining in two hSKP samples treated with different combinations of mTOR activators and inhibitor Rapamycin as well as vehicle DMSO. Prop = 50 μ M Propranolol; MHY = 2 μ M MHY1485; Rapa = 100 nM Rapamycin. (f) Western blots showing the expression of p-Smad2/3 and Smad2 proteins in three hSKP samples treated with different concentrations of SB431542. (g) Ki67 (upper) and SA- β -gal (lower) staining of hSKPs treated with different concentrations of SB431542 and vehicle DMSO. (h) Statistical analysis of Ki67+ cell ratio in hSKPs treated with different concentrations of SB431542 and vehicle DMSO. SB 2= 2 μ M SB431542; SB 10= 10 μ M SB431542. Scale bar = 100 μ m.