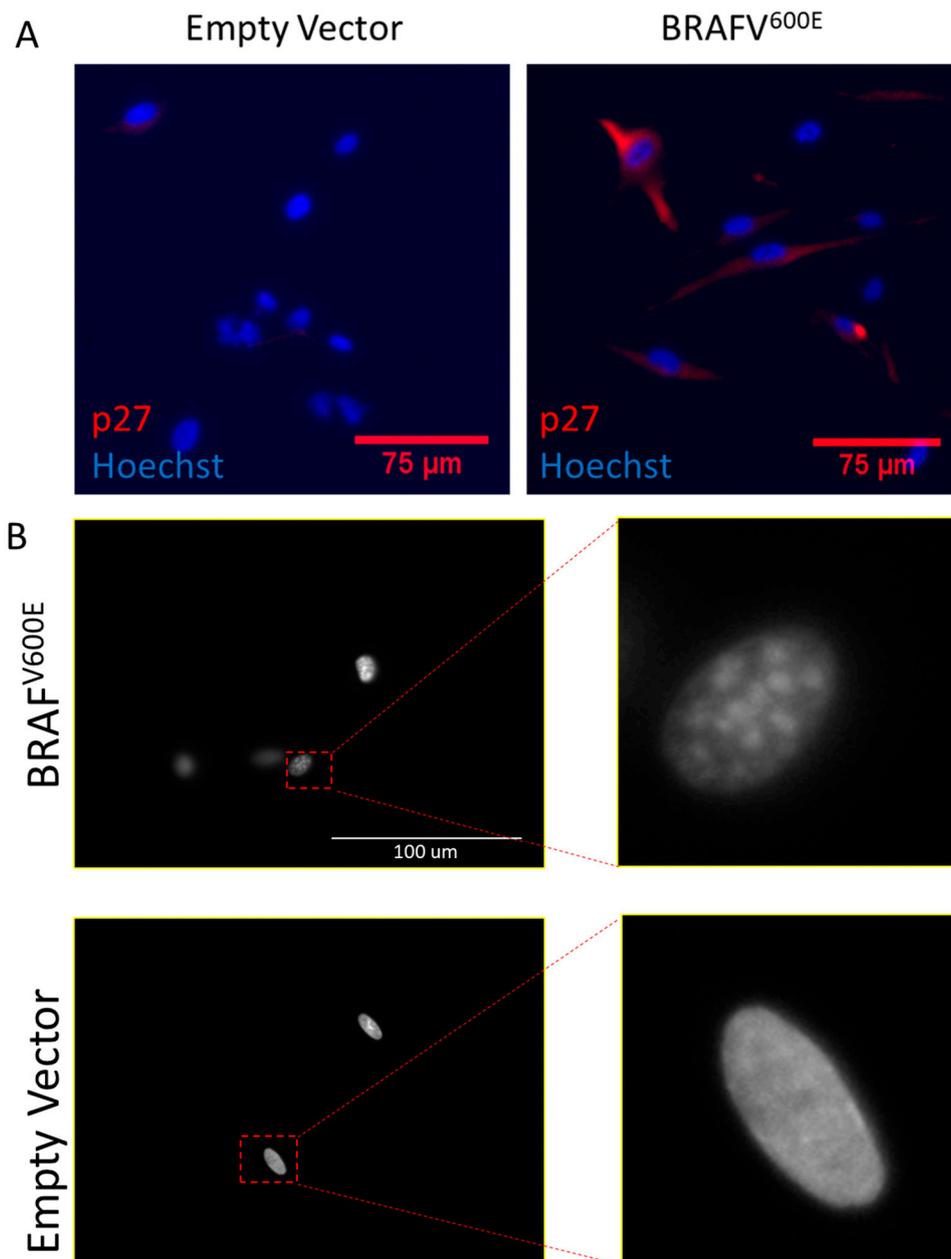
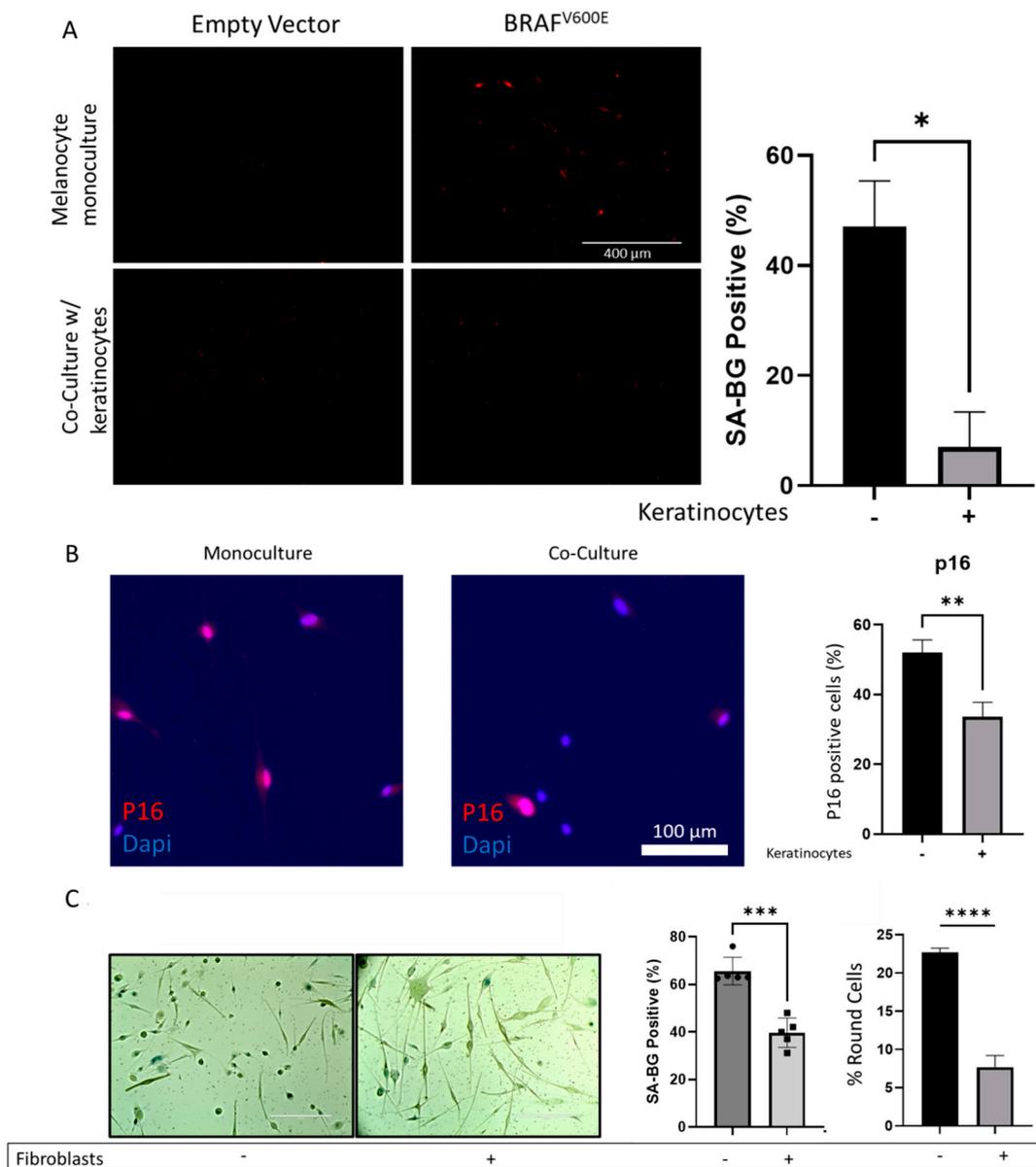


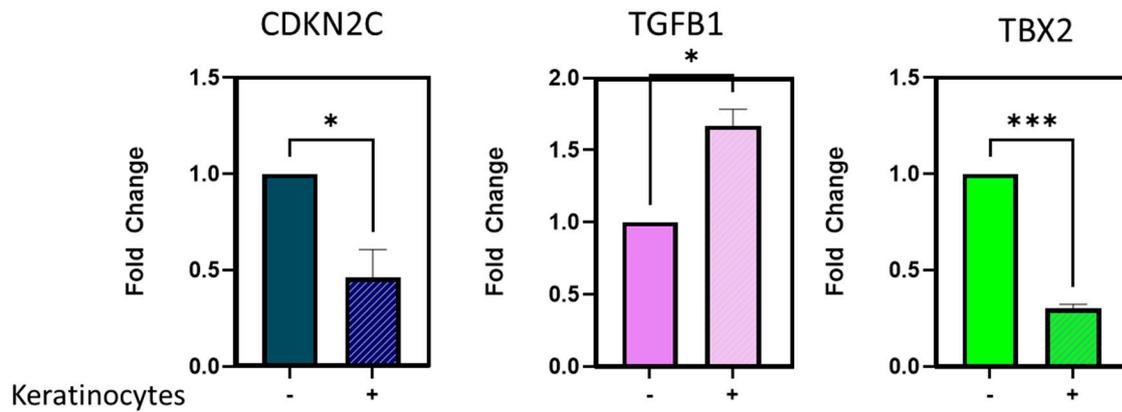
# Role of the Skin Microenvironment in Melanomagenesis: Epidermal Keratinocytes and Dermal Fibroblasts Promote BRAF Oncogene-Induced Senescence Escape in Melanocytes



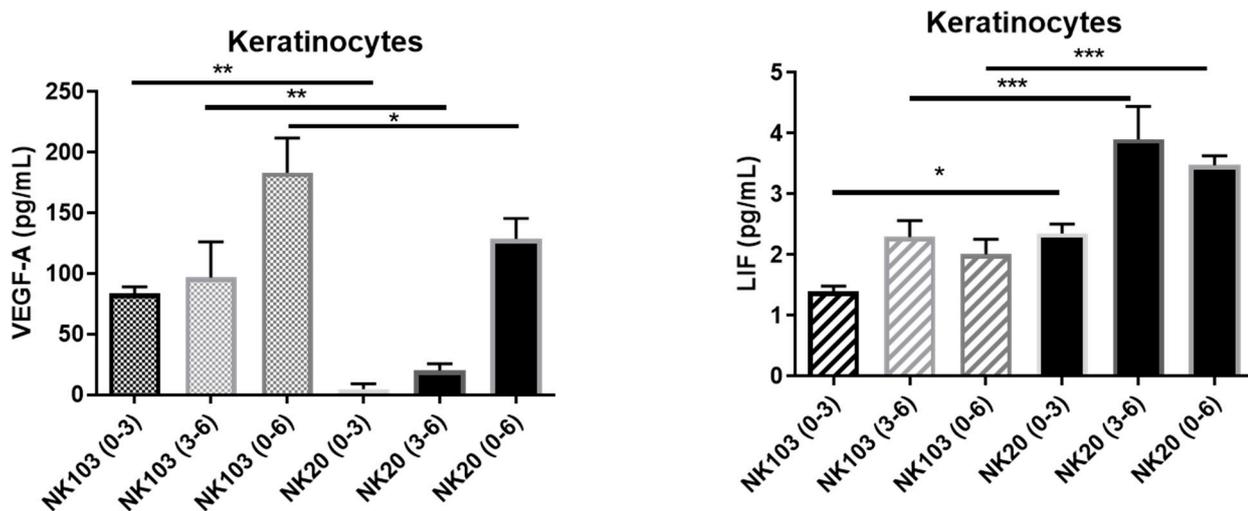
**Figure S1.** (A) BRAF<sup>V600E</sup> transformation induces p27 expression in melanocytes. Left image shows control normal melanocytes and right image shows BRAF<sup>V600E</sup> transformed melanocytes with increased p27 expression as shown by immunofluorescence. (B) Senescence Associated Heterochromatin Foci. BRAF<sup>V600E</sup> melanocytes show punctate staining in nuclei due to the formation of the SAHF. Control melanocytes show a diffused staining of the nuclei.



**Figure S2.** (A) Co-culture of Keratinocytes with BRAFV600E melanocytes. Keratinocytes were co-cultured with empty vector control (Empty Vector) and transformed melanocytes (BRAF<sup>V600E</sup>) seeded on a 24 well plate in a cell culture insert, for 1 week. Figures show SABG analysis of cells with (Co-Culture) and without (Monoculture) keratinocytes. Presence of keratinocytes lowers the number of SABG positive cells. Quantification of senescence on the right image. (B) Co-Culture of Keratinocytes with BRAFV600E melanocytes. Keratinocytes were co-cultured with transformed melanocytes (BRAFV600E) seeded on a 24 well plate in a cell culture insert, for 1 week. Figures p16 immunostaining of cells with (Co-Culture) and without (Monoculture) keratinocytes. Presence of keratinocytes lowers the number of p16 positive cells. Quantification of p16 expression on the right image. (C) Co-Culture of Fibroblasts with BRAFV600E melanocytes. A. Fibroblasts were co-cultured with transformed melanocytes seeded on a 24 well plate in a cell culture insert, for 1 week. Figures show SABG analysis of cells with (+) and without (-) fibroblasts. Presence of fibroblasts lowers the number of SABG positive cells. Quantification of morphology change as percentage of round cells shown on right. \*, \*\*, \*\*\*, and \*\*\*\* denote  $p$ -value < 0.05, 0.01, 0.005, and 0.001 respectively



**Figure S3.** Fold Change of genes CDKN2C, TGFB1, and TBX2 in the presence (+) and absence (-) of keratinocytes. \*, and \*\*\*, denote  $p$ -value  $< 0.05$ , and  $0.005$  respectively.



**Figure S4.** Secretion of VEGF-A and LIF by Keratinocytes. Graph showing amount of VEGF-A and LIF secreted by Caucasian and African American keratinocytes in different intervals. NK103 indicates Caucasian keratinocytes and NK 20 indicates African American keratinocytes. (0-3) represents VEGF-A/LIF in the media built up since seeding of keratinocytes until day 3 without any change of media in between. (3-6) represents VEGF-A/LIF in the media after from day 3 (post media change) to day 6. (0-6) represents VEGF-A/LIF in the media built up since seeding of keratinocytes until 6 days without any change of media in between. \*, \*\*, and \*\*\*, denote  $p$ -value  $< 0.05$ ,  $0.01$ , and  $0.005$  respectively.