

# **Quantitative comparison of the spreading and invasion of radial growth phase and metastatic melanoma cells in a three-dimensional human skin equivalent model**

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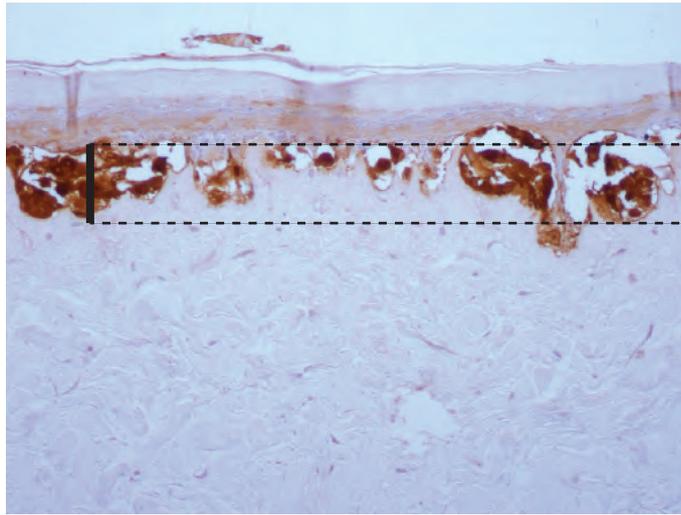
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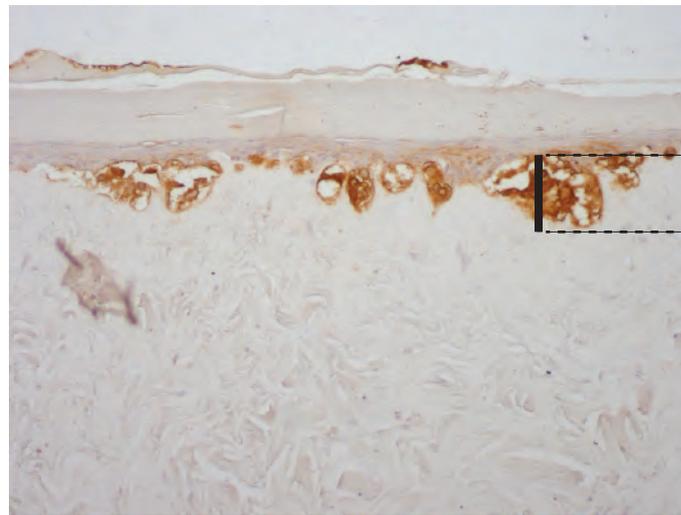
A



MSE: day 9

Depth measured in  $\mu\text{m}$

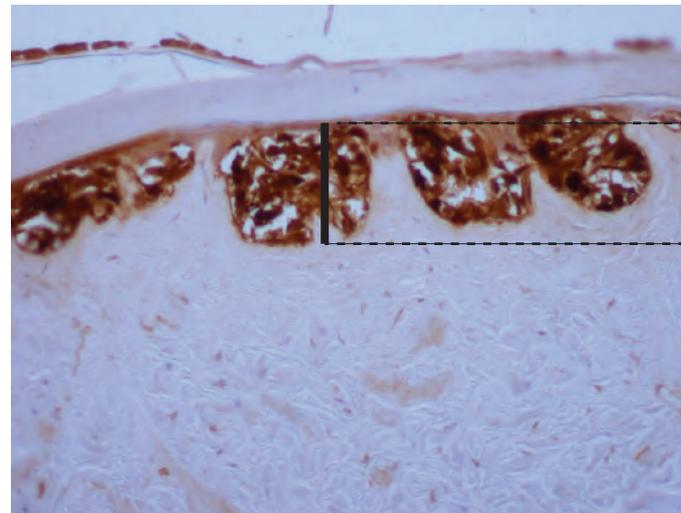
B



MSE: day 15

Depth measured in  $\mu\text{m}$

C



MSE: day 20

Depth measured in  $\mu\text{m}$

**Figure S1: Quantifying depth of melanoma cell invasion.**

(A)-(C) MSE model with SK-MEL-28 melanoma cells (brown) highlighted by S100 at day 9, 15 and 20. The black arrows indicate the depth of melanoma cell invasion measured. Scale bar corresponds to 100  $\mu\text{m}$ .

MTT: day 15

MTT: day 20

A

B

HSE



C

D

MSE: WM35



E

F

MSE: SK-MEL-28



**Figure S2: MTT assay.**

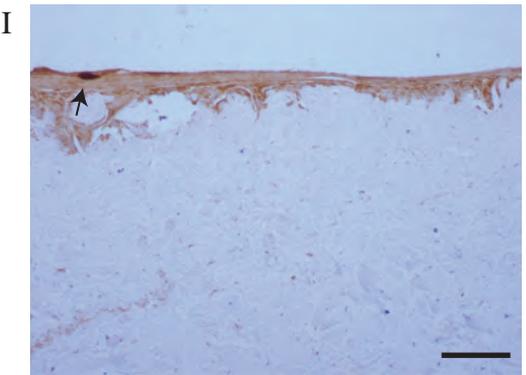
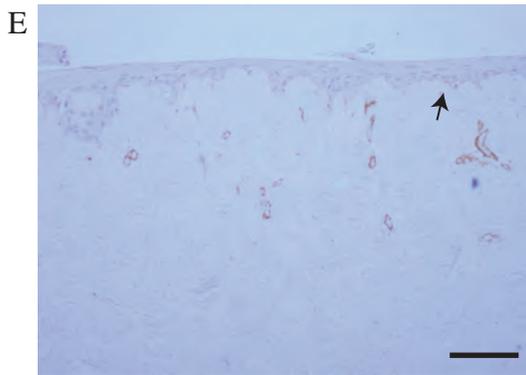
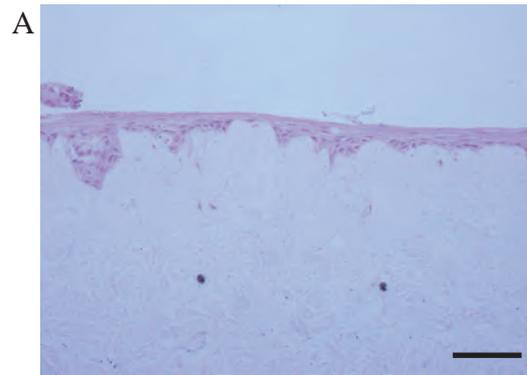
Experimental images of MTT assay shows viable cells (purple) on the HSE model (A) and (B). The MSE model with WM35 melanoma cells is shown in (C) and (D). The MSE model with SK-MEL-28 melanoma cells is shown in (E) and (F). The results in the left column are at day 15, and the results in the right column are at day 20. Scale bar corresponds to 1 mm.

HSE

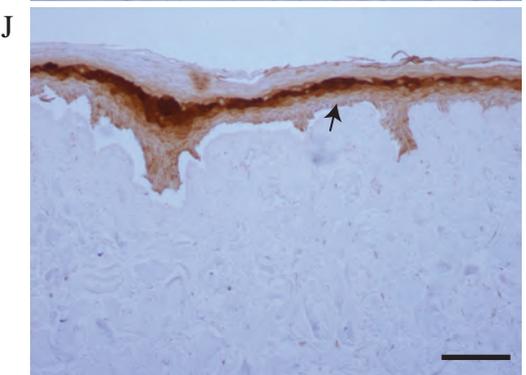
H&E

Col IV

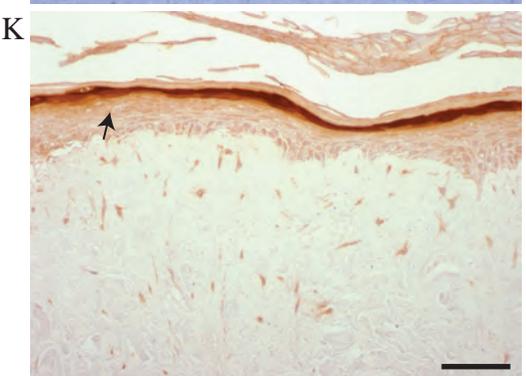
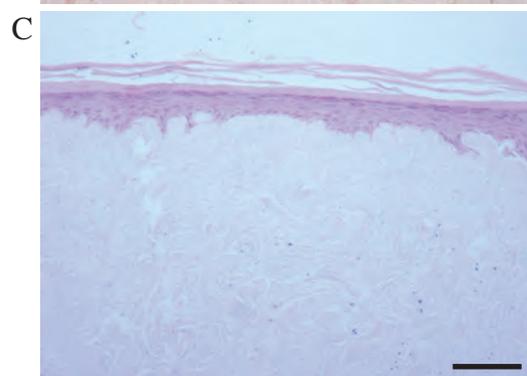
Loricrin



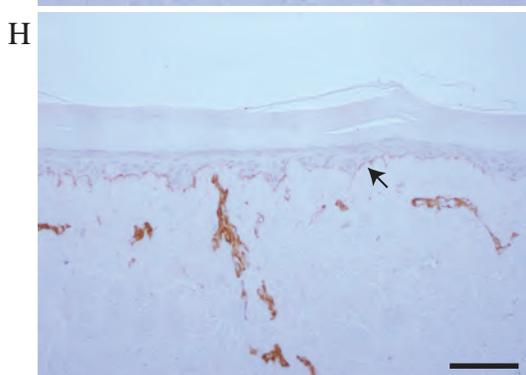
Day 0



Day 9



Day 15



Day 20

**Figure S3: Histological analysis of HSE model.**

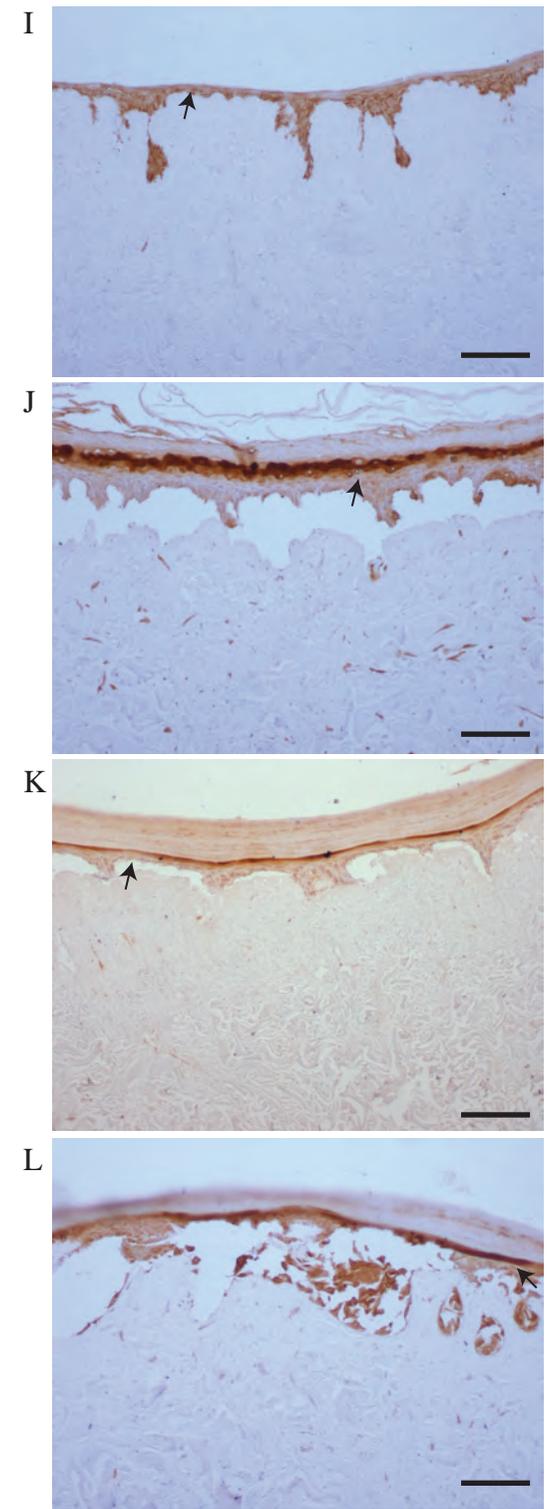
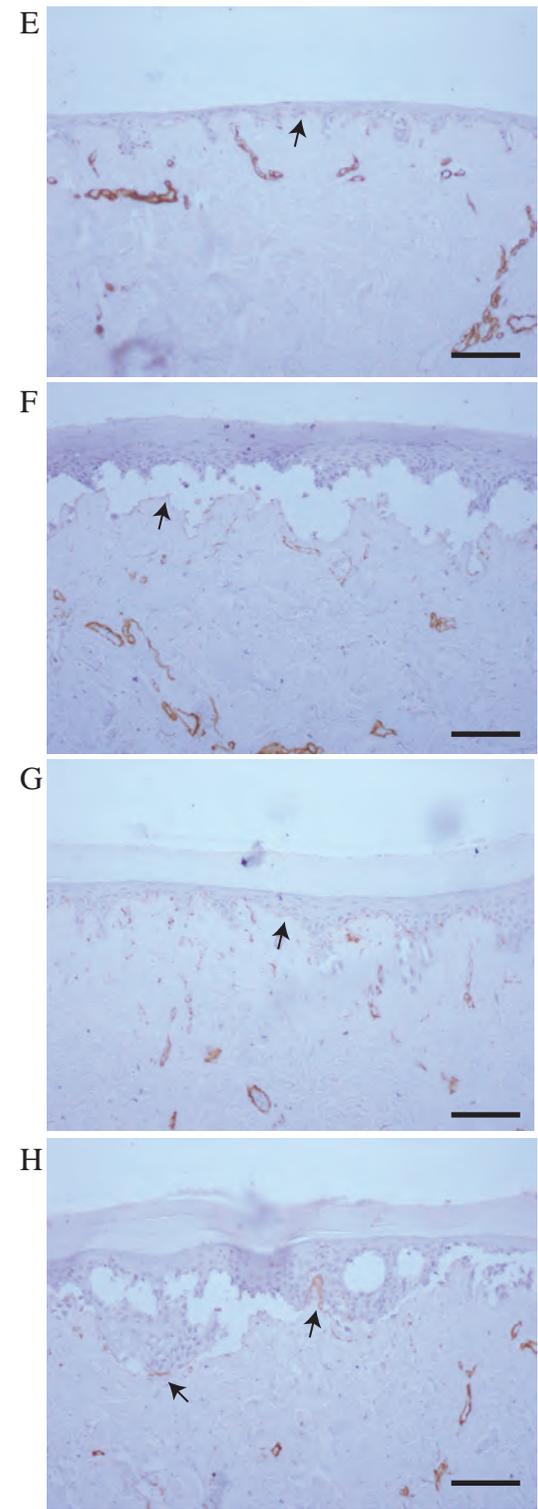
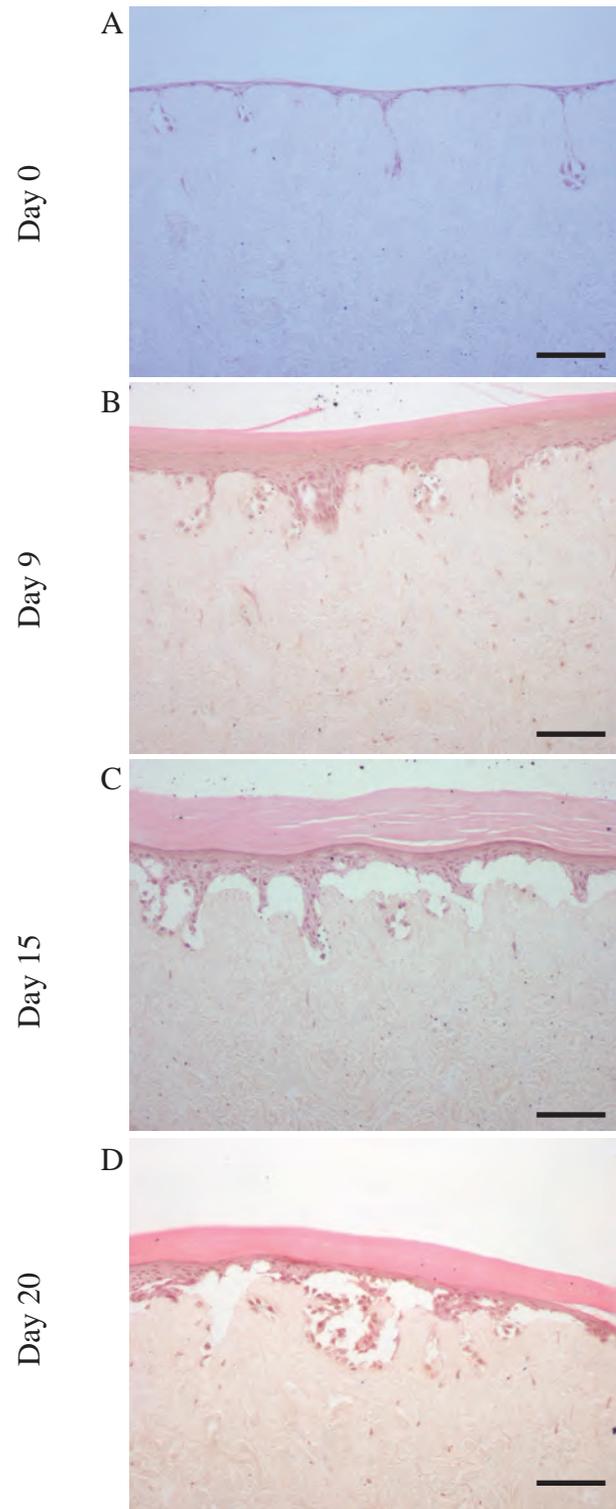
(A)-(D) H&E staining at day 0, 9, 15 and 20. (E)-(H) The basement membrane (brown) highlighted by collagen IV (Col IV) at day 0, 9, 15 and 20. (I)-(L) Terminally differentiating epithelial cells (brown) highlighted by loricrin at day 0, 9, 15 and 20. Black arrows indicate positive staining. Scale bar corresponds to 100  $\mu\text{m}$ .

MSE: WM35

H&E

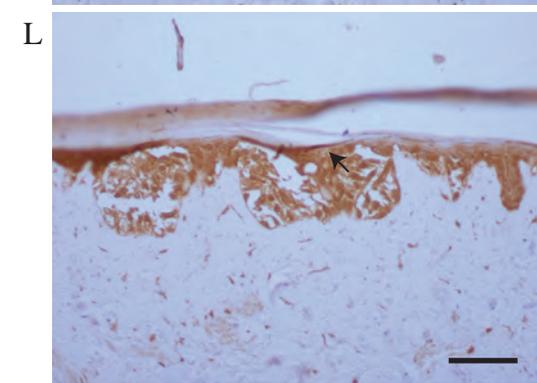
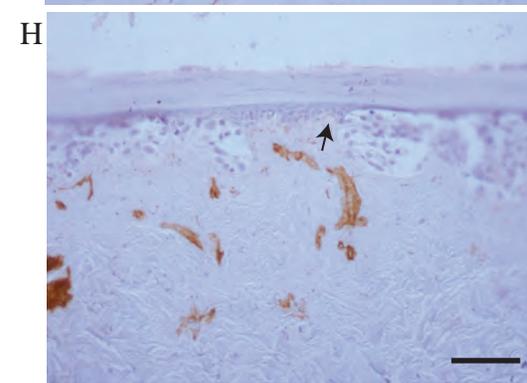
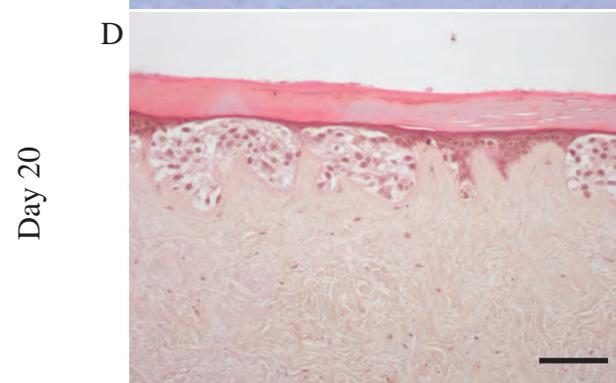
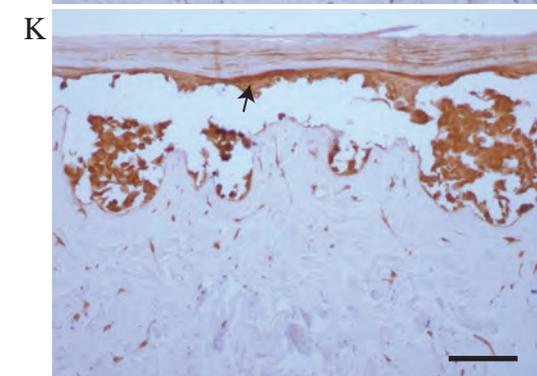
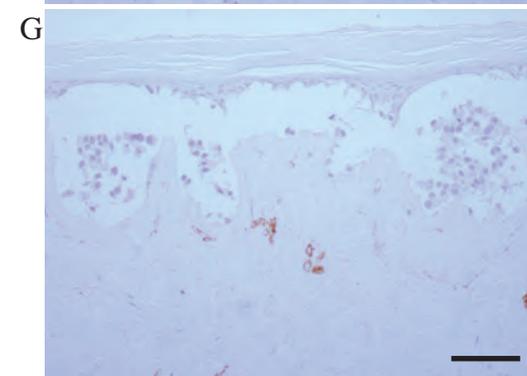
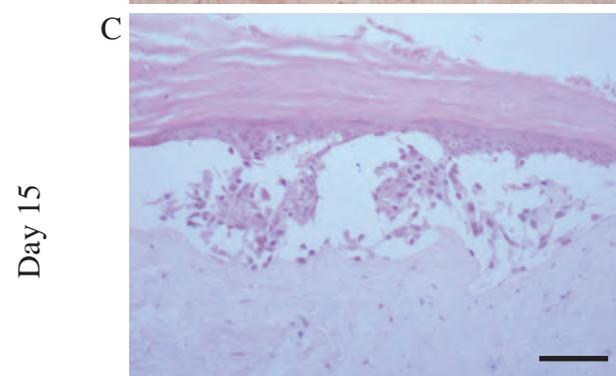
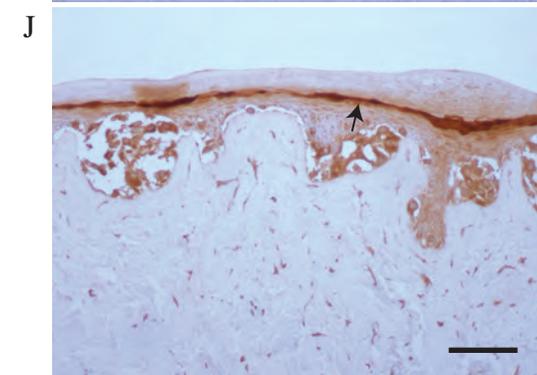
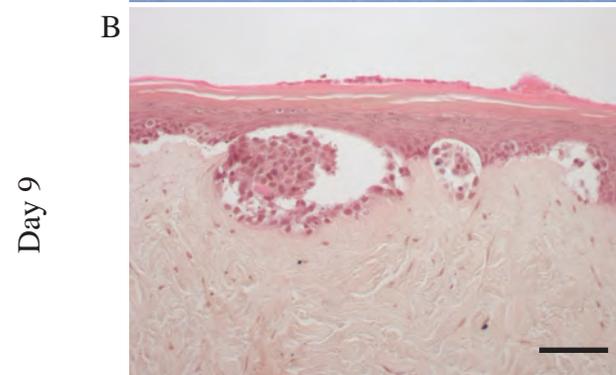
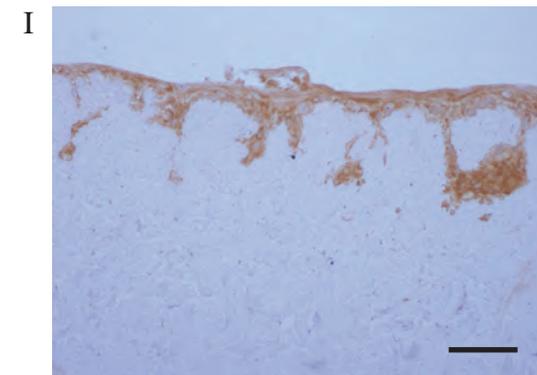
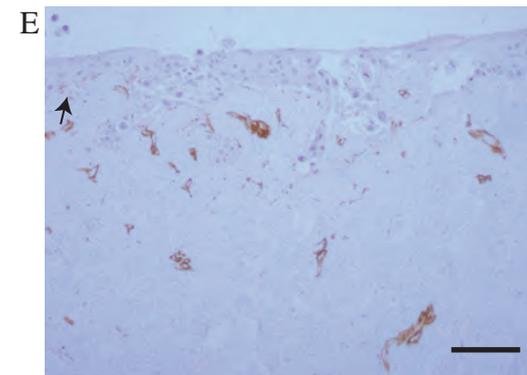
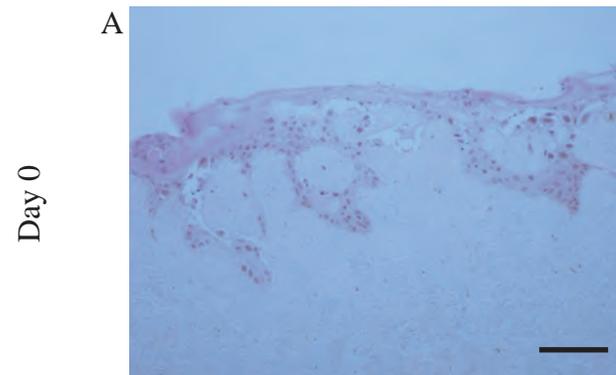
Col IV

Loricrin



**Figure S4: Histological analysis of MSE model with WM35 melanoma cells.**

(A)-(D) H&E staining at day 0, 9, 15 and 20. (E)-(H) The basement membrane (brown) highlighted by collagen IV (Col IV) at day 0, 9, 15 and 20. (I)-(L) Terminally differentiating epithelial cells (brown) highlighted by loricrin at day 0, 9, 15 and 20. Black arrows indicate positive staining. Scale bar corresponds to 100  $\mu\text{m}$ .



**Figure S5: Histological analysis of MSE model with SK-MEL-28 melanoma cells.**

(A)-(D) H&E staining at day 0, 9, 15 and 20. (E)-(H) The basement membrane (brown) highlighted by collagen IV (Col IV) at day 0, 9, 15 and 20. (I)-(L) Terminally differentiating epithelial cells (brown) highlighted by loricrin at day 0, 9, 15 and 20. Black arrows indicate positive staining. Scale bar corresponds to 100  $\mu\text{m}$ .