Histochemistry and Cell Biology

Electronic Supplementary Material

Prolonged overexpression of *Wnt10b* induces epidermal keratinocyte transformation through activating *EGF* pathway

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Supplementary figures and legends

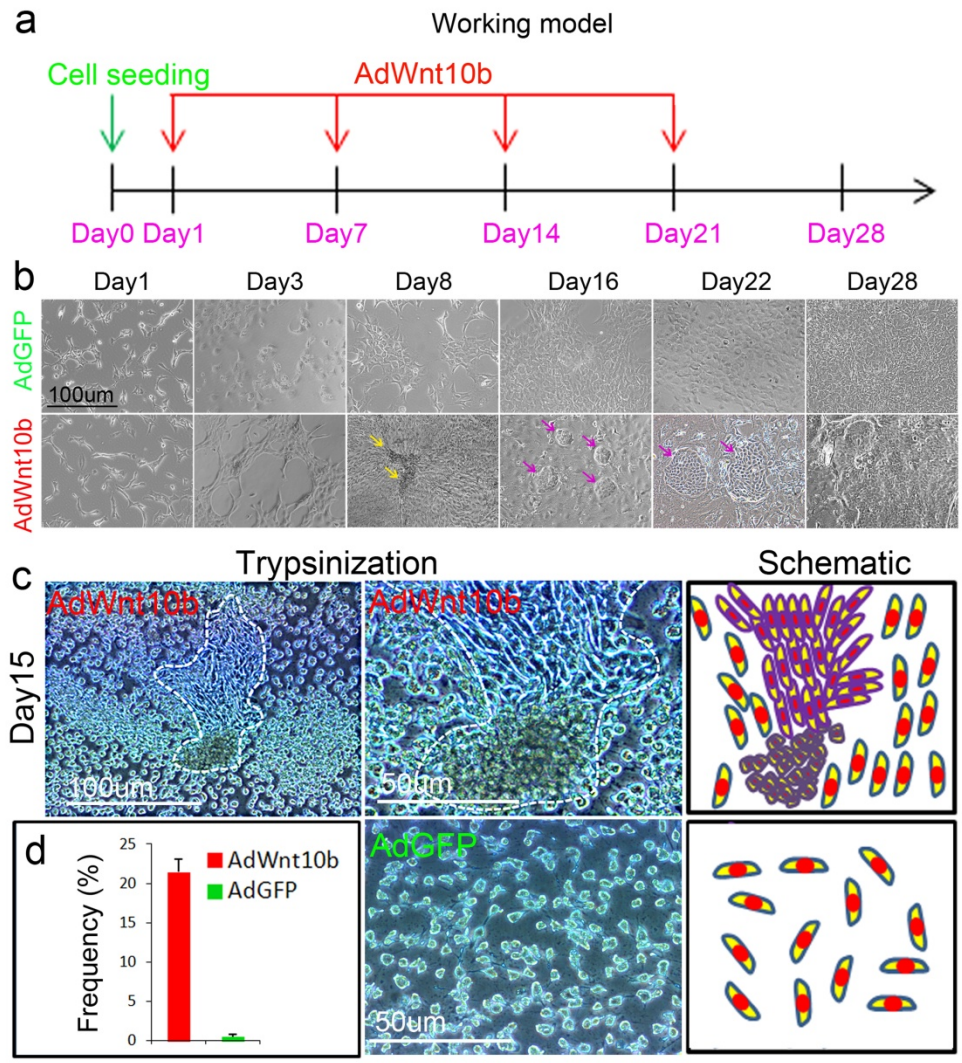

Fig. S1 Stepwise transformation of JB6P- cells after AdWnt10b treatment. a. Working model showed timing of 4 doses of AdWnt10b treatments. b. Low-power view images related to Figure 2a showed stepwise cell transformation during AdWnt10b treatments. c. Phase-contrast microscopy and schematic drawing showed different cell shapes digested with trypsin at day 15 after treatment with 3 doses of AdWnt10b. d. The incidence of cell

transformation frequency was ~~almost 100%~~about 21.3% in supernatant treatment assay. n＞5. P＜0.05.

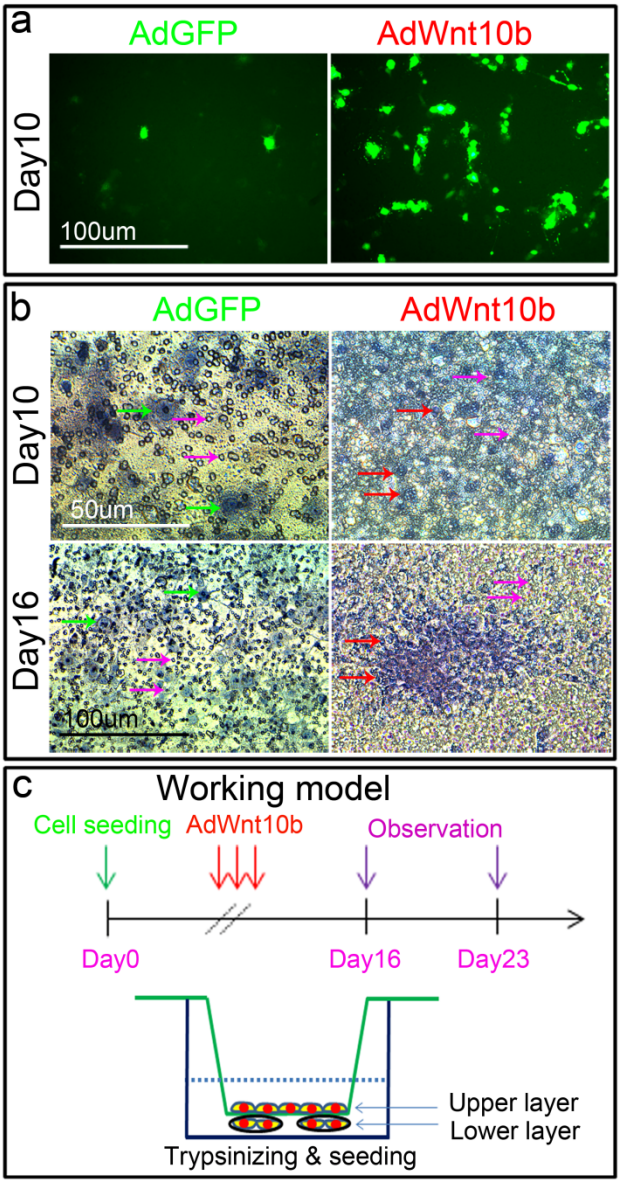

Fig. S2 Invasive ability of JB6P- cells was enhanced after 3 doses of AdWnt10 treatment. a. Fluorescent images showed more cells were invaded into the lower part of the transwell culture system in AdWnt10b-treated group 10 days after infection. b. H&E staining revealed

more cells were invaded into the lower part of the transwell culture system at day 10 and form cluster at day16 after 2 or 3 doses of AdWnt10b treatment (Green arrows, AdGFP-induced cells; purple arrows, transwell holes; red arrows, AdWnt10b-induced cells). c. Working model showed timing and culture system for cell seeding, AdWnt10b application and observation.

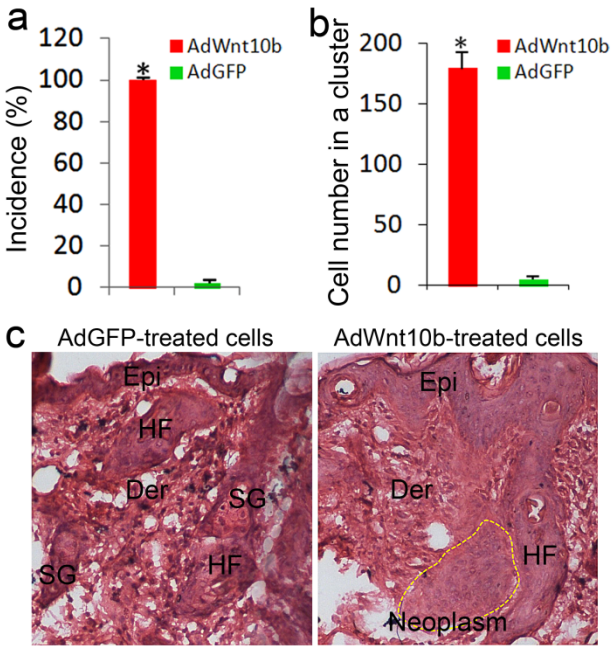

Fig. S3 ~~Anchorage-independent growth~~ Neoplasm formation of AdWnt10b-treated cells. a. The incidence of cell cluster was 100% in AdWnt10b-treated group. b. The average cell number in one cluster was about 170. c. Neoplasm was observed in nude mouse skin in AdWnt10b-treated group at day 12 after subcutaneous injection. Der, dermis; Epi, epidermis; HF, hair follicle; SG, sebaceous gland. n＞5. *P＜0.05.

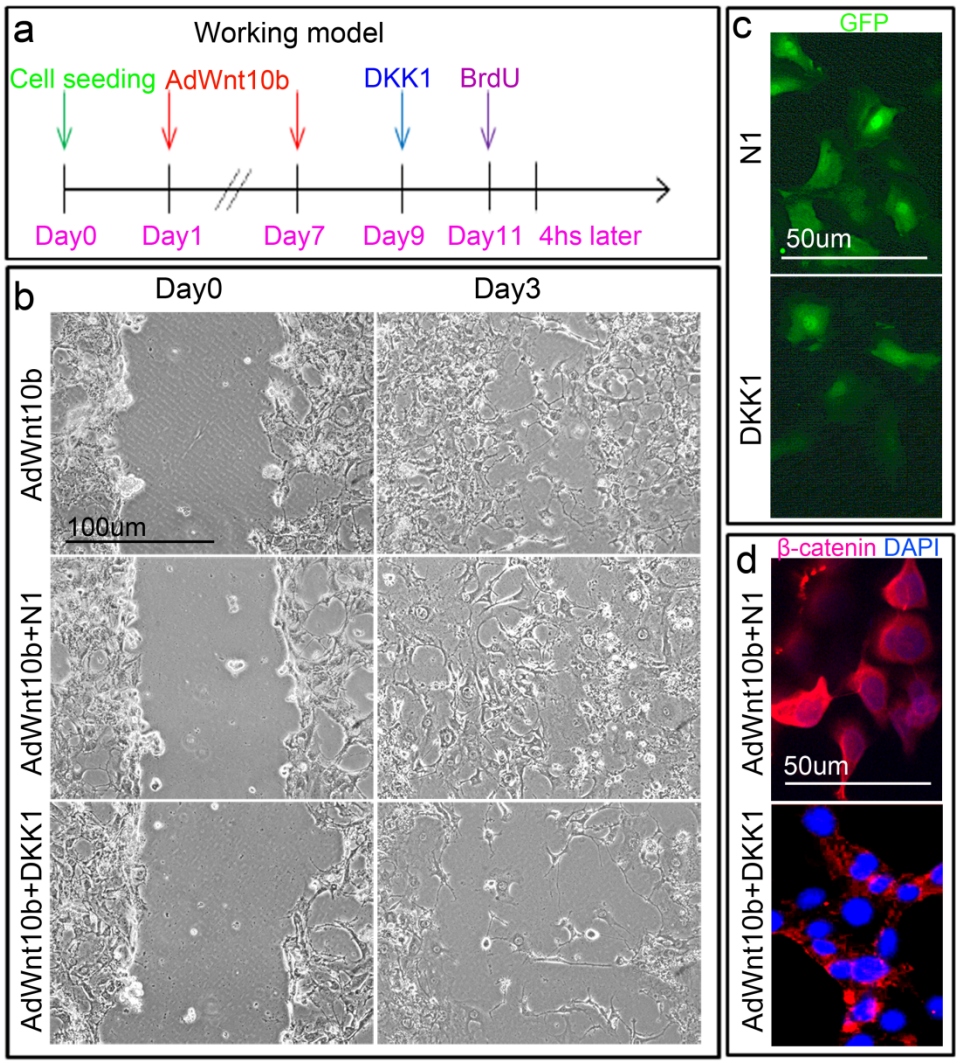

Fig. S4 Decrease of cell migration and Wnt pathway activation after sequential AdWnt10b+DKK1 treatment. a. Working model showed timing of cell seeding, AdWnt10b and Dkk1 administrations, BrdU application and detection. b. Cell migration rate was decreased in AdWnt10b+DKK1-treated group, when compared to the AdWnt10b or AdWnt10b+N1-treated groups. c. GFP revealed authenticity of Dkk1 plasmid transfection. d. β-catenin was only expressed in the cytoplasm of JB6P- cells after treatment with AdWnt10b+DKK1, when compared to the AdWnt10b+N1 group, which showed fewer nuclear localization of β-catenin.

Supplementary table

Gene name		Primer sequences (5'-3')
AP-1	Fwd	TCCCCTATCGACATGGAGTC
AP-1	Rev	GCTTAAGCTGTGCCACCTGT
Erk1	Fwd	AGCCCCAGAGATCATGCTTA
Erk1	Rev	CGGGCCTTCATGTTAATGAT
Erk2	Fwd	ATCTGTGACTTTGGCCTTGC
Erk2	Rev	GCTTTCCTGGGAAGATAGGC
IKKb	Fwd	CTCCCTGACAAGCCTGCTAC
IKKb	Rev	TTCCTCAGCTGGAAGAAGGA
p65	Fwd	TTCCTCAGCCATGGTACCTC
p65	Rev	ACTCCTGGGTCTGTGCTGTT
EGF	Fwd	CCCAGGCAACGTATCAAAGT
EGF	Rev	GGTCATACCCAGGAAAGCAA
E-cadherin	Fwd	CGGAGAGGAGAGTCGAAGTG
E-cadherin	Rev	CATGCTCAGCGTCTTCTCTG
Gsk3β	Fwd	CAAGCAGACACTCCCTGTGA
Gsk3β	Rev	TGAAACATTGGGCTCTCCTC
β-catenin	Fwd	TGCAGAAAATGGTTGCTTTG
β-catenin	Rev	CCTTCAGCACTCTGCTTGTG
Wnt10b	Fwd	ATACCCACAACCGCAACTC
Wnt10b	Rev	CACGATAAACCCTAGACAGAAA
DKK1	Fwd	CTGAAGATGAGGAGTGCGGCTC
DKK1	Rev	GGCTGTGGTCAGAGGGCATG
Mmp2	Fwd	CAAGTTCCCCGGCGATGTC
Mmp2	Rev	TTCTGGTCAAGGTCACCTGTC
Mmp7	Fwd	CTGCCACTGTCCCAGGAAG
Mmp7	Rev	GGGAGAGTTTTCCAGTCATGG
Mmp9	Fwd	CTGGACAGCCAGACACTAAAG
Mmp9	Rev	CTCGCGGCAAGTCTTCAGAG
Mmp12	Fwd	GAGTCCAGCCACCAACATTAC
Mmp12	Rev	GCGAAGTGGGTCAAAGACAG
Timp1	Fwd	GCAACTCGGACCTGGTCATAA
Timp1	Rev	CGGCCCGTGATGAGAAACT

Supplementary table 1. Primers were used in this study. Fwd, forward primer; Rev, reversed primer.