Fig. S1. Wound closure, smooth muscle cell differentiation, inflammation and scar formation in control and K5-R1/R2 mice.

(A) Sections from 5-day wounds were stained with H/E. Wound closure was determined using histomorphometry. An average of 2 wounds each from at least 6 mice per genotype were analyzed. Bars represent mean ± s.e.m. (B) Different components of a 5-day wound are shown schematically. The region, where myofibroblasts were analyzed by staining with an antibody against α smooth muscle actin (SMA), is indicated by a green rectangle, the region were neutrophils were analyzed by staining with an antibody against Ly-6G is indicated by a red rectangle. (C) Sections from 5-day wounds from control and K5-R1/R2 mice were analyzed by immunofluorescence for the presence of cells expressing α smooth muscle actin (green). Representative sections are shown. α-SMA positive myofibroblasts at the wound edge are indicated with an arrow. The border between hyperproliferative wound epidermis and dermis/granulation tissue is indicated with a dotted line. Staining in the wound epidermis results from unspecific autofluorescence. (D) Sections from 5-day wounds from control and K5-R1/R2 mice were analyzed by immunofluorescence for the presence of Ly-6G positive neutrophils (red dots). Representative sections are shown. (E) Sections from 13-day wounds were stained using the Masson Goldner protocol. Deposited collagen appears green. An overview of one wound halve is shown on the left hand side and a higher magnification of the area of granulation tissue indicated by the rectangle is shown on the right hand side. All scale bars represent 200 μm.
Fig. S2. The migratory capacity is reduced in immortalized keratinocytes from FGFR1 and FGFR2 deficient mice. (A) Immortalized keratinocytes from control and K5-R1/R2 mice were analyzed in a transwell assay for their migratory capacity. The migrated cells on the bottom side of the membrane were counted 24h after seeding. The graph shows cells counted per field. Bars represent mean ± s.e.m. (B) Immortalized keratinocytes from control and K5-R1/R2 mice were grown to confluency on collagen I coated dishes. A scratch wound was inserted into the monolayer, and pictures were taken immediately after scratch wounding (0h) and 21h later.
Fig. S3. Loss of FGFR1 and FGFR2 reduces velocity and impairs directional migration of cultured keratinocytes at the leading edge of the scratch and further behind. Immortalized keratinocytes from control and K5-R1/R2 mice were subjected to scratch wounding and analyzed by live cell imaging for 13 h. Eight cells from a minimum of 5 movies were analyzed for each genotype – 4 cells from the leading edge (front row) and 4 cells located further behind (fifth line). All results were reproduced with an independent cell line from control and K5-R1/R2 mice. (A) Schematic illustration of parameters analyzed for the quantification of migration. The migrating cells were analysed for (B) velocity, (C) displacement (linear distance between starting and end point; “b” in (A)), (D) persistence coefficient (“b”/“a” in (A)), and (E) perpendicular movement (“c” in (A)). Bars represent mean +/- s.e.m. Note the similar migratory behaviour of cells in the front row and in the fifth row, with exception of velocity, where a difference between genotypes was only observed for cells in the front row.
Fig. S4. FGFR signaling controls expression of paxillin and FAK, but not of integrins, laminin-332 or MMPs in keratinocytes

(A) Total lysates from immortalized (left panel) and wild-type keratinocytes (right panel) of control and K5-R1/R2 mice (different cell lines or isolates) were analyzed by western blotting for the expression of the γ2-chain of laminin-332 (LM-γ2). GAPDH was used as a loading control. (B) Immortalized keratinocytes from control and K5-R1/R2 mice were seeded into non-coated culture dishes. Forty-five minutes after seeding cells were fixed, permeabilized, and analyzed by immunofluorescence using an antibody against the γ2-chain of laminin-332 (green). They were counterstained with rhodamine-conjugated phalloidin (red). Scale bars left panels: 100 μm; right panels 20 μm. (C) Protein lysates from primary (upper panels) and immortalized keratinocytes (lower panels) from control and K5-R1/R2 mice were analyzed by western blotting for the expression of the integrin α6 subunit (ITGα6). Probing of the membrane with an antibody against GAPDH was used as a loading control for the primary cells and tubulin was used for the cell lines. (D) Protein lysates from primary keratinocytes from K5-R1/R2 and control mice were analyzed for levels of total and phosphorylated paxillin and FAK or β-actin (loading control). Bars represent mean ± s.e.m. (E) Expression and activation of MMP9 and MMP10 was analyzed by casein zymography of cell culture supernatants of immortalized keratinocytes from control and K5-R1/R2 mice. A representative gel is shown. The experiment was performed in triplicate with 3 independent cell lines per genotype. (F) RNA was isolated from the epidermis of P12 K5-R1/R2 mice and age- and sex-matched control animals and analyzed for the expression of paxillin and Fak by qRT-PCR. Expression of the housekeeping gene rps29 was used for normalization. Expression levels in vehicle-treated cells were arbitrarily set to 1. N=5 control mice and 6 K5-R1/R2 mice.
Movie 1. Migration of control keratinocytes. Immortalized keratinocytes from control mice were subjected to scratch wounding. Their movement was subsequently recorded by live cell imaging for 48 h. Representative movies of the healing process of scratch wounds from control keratinocytes are shown. The experiments were repeated 3 times with 2 different cell lines.

Movie 2. Loss of FGFR1 and FGFR2 reduces velocity and impairs directional migration of cultured keratinocytes. Immortalized keratinocytes from K5-R1/R2 mice were subjected to scratch wounding. Their movement was subsequently recorded by live cell imaging for 48 h. Representative movies of the healing process of scratch wounds from K5-R1/R2 keratinocytes are shown. The experiments were repeated 3 times with 2 different cell lines.