**4-aminopyridine promotes accelerated skin wound healing**

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**Supplementary Information**

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**Supplementary Fig. S1. 4-AP induced neo-angiogenesis and neuronal peptide wound healing and did not alter keratinocyte K10 expression. (A)** Keratin 10 protein expression in healed epidermis by immunofluorescence. 4-AP treatment did not cause any change in expression of keratinocyte K10 expression. Scale bars = 50 µm. **(B)** Percent of K10+ cells in control and 4-AP treated skin wounds at day 14. Mean ± SEM; N = 4 animals wound tissue/group and \*P = 0.01 to 0.05, \*\*P = 0.01 to 0.001, \*\*\*P < 0.0002, and \*\*\*\*P < 0.0001 unpaired t-test. **(C)** Immunofluorescence staining of control and healed wound sections for pan-neuronal marker PGP-9.5 (red) and nuclear stain DAPI (blue). Scale bars = 20 μm. **(D)** Quantification of PGP-9.5 protein expressing cells showed significantly increased PGP-9.5 intensity in the 4-AP treated group compared to the saline treated group at day 14. PGP 9.5 in 4-AP-treated mice was not significantly different from seen in uninjured (control) tissue. Mean ± SEM; N = 6 animal wound tissues/group and 4 control tissue, \*P = 0.01 to 0.05, \*\*P = 0.01 to 0.001, \*\*\*P < 0.0002, and \*\*\*\*P < 0.0001, unpaired t-test. **(E)** CD31 protein expression in healed skin by immunofluorescence. Scale bars = 100 µm. **(F)** Quantification of CD31 staining intensity, 4-AP treated group showed higher intensity compared to saline control group. Mean ± SEM, N = 6 animals wound tissue/group and \*P = 0.01 to 0.05, \*\*P = 0.01 to 0.001, \*\*\*P < 0.0002, and \*\*\*\*P < 0.0001 unpaired t-test.

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**Supplementary Fig. S2. Isolation and characterization of human skin derived primary keratinocytes, Schwann cells, and fibroblasts.** (A) Keratinocytes were characterized using keratin 14 (K14, a marker of proliferative keratinocytes) and K10 (keratin 10, a marker of keratinocyte differentiation marker). Scale bars = 100µm. **(B)** Schwann cells were identified using S100 (Schwann cells marker) and p75-NTR (nerve growth factor receptor marker). Scale bars = 50 µm. **(C)** Fibroblasts were characterized using vimentin (fibroblast marker) and α-smooth muscle actin (fibroblast differentiation, myofibroblast marker). Scale bars, 50 µm. **(D-F)** Cell viability using MTT assay with different concentrations of 4-AP (ranging from 1 to 10000 µM) for **(D)** Keratinocytes, **(F)** Schwann cells and **(F)** fibroblast. Mean ± SEM, N=3 replicates/concentration of 4-AP.

 **Supplementary Fig. S3. Effect of 4-AP on cultured keratinocytes.** Primary keratinocytes were exposed to 4-AP or no treatment for 24 hours and co-immunostained using antibodies against keratin 14 (proliferation-K14-green), keratin 10 (differentiation-K10-red) or keratin 17 (hyperproliferation-K17-yellow). DAPI (blue) was used as nuclear counterstaining. Scale bars = 100 µm.

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**Supplementary Fig. S4. Effect of 4-AP exposure on primary dermal fibroblasts.** **(A)** Representative images of *in-vitro* fibroblast scratch assays with 4-AP and vehicle control at indicated time points. Scale bar = 100 µm. **(B)** The relative percentage of wound closure was calculated as the ratio of the remaining wound gap at the given time point compared to time 0. Data expressed as means ± SEM, N = 4 wound scratch replicates/group, and \*P = 0.01 to 0.05, \*\*P = 0.01 to 0.001, \*\*\*P < 0.0002, and \*\*\*\*P < 0.0001 one-way ANOVA Sidak's multiple comparisons test. **(C)** Co-immunostained dermal fibroblasts exposed to 4-AP or no treatment for 72 hours and immunostained using antibodies against the fibroblast marker vimentin- (red), or the myofibroblast marker α-SMA (green). DAPI (blue) was used as nuclear counterstaining.

**M:\Dr. Elfar Lab work\Wound healing\Manuscript- Wound healing\Mark noble\Nature communication\For submission\Supp figures\Supp Fig 5.tif Supplementary Fig. S5. Effect of 4-AP exposure on primary dermal Schwann cell in scratch wound healing assays.** **(A)** Representative images of *in-vitro* Schwann cells scratch assays with 4-AP and vehicle controls at indicated time points. Scale bar =100 µm. **(B)** The relative percentage of wound closure was calculated as the ratio of the remaining wound gap at the given time point compared to time 0. Mean ± SEM, N = 5 wound scratch replicates/group, and \*P = 0.01 to 0.05, \*\*P = 0.01 to 0.001, \*\*\*P < 0.0002, and \*\*\*\*P < 0.0001 one-way ANOVA Sidak's multiple comparisons test. **(C)** Co-immunostaining of dermal Schwann cells exposed to 4-AP or no treatment for 72 hours and immunostained against SC markers including the Schwann cell marker S100 (green), a de-differentiation marker p75-NTR (red) and myelin basic protein (MBP-yellow). DAPI (blue) was used as nuclear counterstaining. Scale bars, 100 µm. **(D - F)** A representative western blot and normalized integrated densities for SOX10, p75-NTR, NGF and GAPDH.

**Supplementary Movies**

**Supplementary Movie 1.** An example of time-lapse phase contrast images depicting the migration of keratinocytes without treatment (control) during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 2.** An example of time-lapse phase contrast images depicting the migration of keratinocytes after 4-AP treatment during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 3.** An example of time-lapse phase contrast images depicting the migration of fibroblasts without treatment (control) during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 4.** An example of time-lapse phase contrast images depicting the migration of fibroblasts after 4-AP treatment during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 5.** An example of time-lapse phase contrast images depicting the migration of Schwann cells without treatment (control) during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 6.** An example of time-lapse phase contrast images depicting the migration of Schwann cells after 4-AP treatment during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 7.** An example of time-lapse phase contrast images depicting the migration of co-cultured keratinocytes and Schwann cells without treatment (control) during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 8.** An example of time-lapse phase contrast images depicting the migration of co-cultured keratinocytes and Schwann cells after 4-AP treatment during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 9.** An example of time-lapse phase contrast images depicting the migration of co-cultured keratinocytes and fibroblasts without treatment (control) during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.

**Supplementary Movie 10.** An example of time-lapse phase contrast images depicting the migration of co-cultured keratinocytes and fibroblasts after 4-AP treatment during wound scratch closure. Images were recorded every one hour. Scale bar = 100 µm.