LETTERS

Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-γ and PTEN

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Wound healing is essential for maintaining the integrity of multicellular organisms. In every species studied, disruption of an epithelial layer instantaneously generates endogenous electric fields, which have been proposed to be important in wound healing[1–3]. The identity of signalling pathways that guide both cell migration to electric cues and electric-field-induced wound healing have not been elucidated at a genetic level. Here we show that electric fields, of a strength equal to those detected endogenously, direct cell migration during wound healing as a prime directional cue. Manipulation of endogenous wound electric fields affects wound healing *in vivo*. Electric stimulation triggers activation of Src and inositol–phospholipid signalling, which polarizes in the direction of cell migration. Notably, genetic disruption of phosphatidylinositol-3-OH kinase-γ (PI(3)Kγ) decreases electric-field-induced signalling and abolishes directed movements of healing epithelium in response to electric signals. Deletion of the tumour suppressor phosphatase and tensin homolog (PTEN) enhances signalling and electrotactic responses. These data identify genes essential for electrical-signal-induced wound healing and show that PI(3)Kγ and PTEN control electrotaxis.

Endogenous wound electric fields were determined first more than 150 yr ago by the German physiologist Emil Du-Bois Reymond[4]. Such electric fields are generated when the epithelial layer is cut and the lesion short-circuits the transepithelial potential difference[1,5–9]. Using various techniques, we confirmed consistent and sustained outward electric currents at wounds in human skin and in rodent cornea and skin (Fig. 1a, b). A large outward current of $4\,\mu A\,cm^{-2}$ was measured at the wound edges of rat cornea and human skin. This current gradually increased to $10\,\mu A\,cm^{-2}$ and persisted at $4–8\,\mu A\,cm^{-2}$. The direction and magnitude of the current was independent of wound size and the current vector (the flow of positive charge) was directed towards the wound centre (Fig. 1a, b).

To test directly the effects of the electric signal on cell movement in wound healing, cell migration was monitored in monolayer epithelial cultures. In control monolayer corneal epithelium without an applied electric field (default healing), cells moved into the wounds in a coordinated manner[10]. When an electric field was applied with a polarity that opposed the default healing direction, the movement of the epithelium followed the direction of the electric signal and the wound opened up (Fig. 1c; Supplementary Fig. 1a). Reversal of the electrical polarity closed the wound (Fig. 1c, 99–204 min; Supplementary Movie 1). An electric field of $12.5\,mV\,mm^{-1}$ with the cathode in the wound resulted in a significant increase ($P = 0.046$) in the distance of cell movement into the wound. Increasing the field strength increased the speed of epithelial movement into the wound and this migration rate reached a maximum at $\sim 100–200\,mV\,mm^{-1}$ (Supplementary Fig. 1b). This field strength is comparable to the strength of endogenous wound electric fields (that is, $42–100\,mV\,mm^{-1}$), measured experimentally in animals and in humans[1,11]. In addition, neutrophils and dermal fibroblasts that are also crucial for wound healing showed evident voltage- and time-dependent electrotactic responses (Supplementary Figs 2 and 3, and Movies 2 and 3). Notably, electrical cues also guide migration of stratified epithelium and control healing rates in a cornea whole-organ culture model[12] (Supplementary Fig. 4 and Movie 4). Thus, electrical signals are predominant directional cues that guide and stimulate the migration of inflammatory cells, fibroblasts and epithelial cells in wound healing.

To test the role of the endogenous electric field in wound healing, we manipulated transepithelial ion transport in epithelial wounds of rat cornea[7,13,14]. In temporal and spatial maps of endogenous electric currents, Cl^- and Na^+ are the main components of electric currents in rat corneal wounds[7] (Fig. 1a). Invariably, enhancing the ion flow increased endogenous wound electric fields and wound healing. $AgNO_3$, which increases Cl^- efflux and Na^+ influx in corneal epithelium, significantly amplified the transcorneal potential difference ($P = 1.30 \times 10^{-7}$) (Supplementary Fig. 5a) and endogenous wound electric field ($P = 0.042$) (Supplementary Fig. 5b), resulting in augmented corneal wound healing *in vivo* (Supplementary Fig. 5c, d). By contrast, furosemide, which inhibits Cl^- efflux, significantly decreased the transcorneal potential difference ($P = 6.72 \times 10^{-8}$) resulting in a decreased endogenous wound electric field ($P = 0.01$), and impaired corneal wound healing (Supplementary Fig. 5). These results, together with our data on electric-field-regulated directional nerve growth and cell proliferation[7,13,14], suggest that endogenous transcellular potentials at wounds have an important role not only *in vitro* but also *in vivo*. The specificities of the agents used to manipulate endogenous electrical fields are discussed in the Supplementary Information.

How are electric migration cues relayed into cellular responses? To answer this question, we analysed whether electric fields induce specific signalling cascades similar to those observed in chemotactic cell migration[15–22]. Intriguingly, exposure of both keratinocytes and neutrophils to electric fields in serum-free medium induced rapid and sustained phosphorylation of extracellular-signal-regulated kinase (ERK), p38 mitogen-activated kinase (MAPK), Src and Akt on Ser 473 (Fig. 1d). Phosphorylation of the Janus kinase JAK1 remained unchanged, indicating that electric currents activate only defined signalling pathways. Next, we examined the distribution of

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activated Src and Akt in electrotactic cells because such intracellular signals are polarized in chemotaxis by means of activation of PI(3)K signalling at the leading edge, and lateral and back inhibition by PTEN in *Dictyostelium* or Rho in neutrophils[15–22]. To test whether, as in classical chemotaxis, electric-field-induced signalling is polarized, we stained cells to assess the distribution of activated Src kinase. In electrotactic keratinocytes, phosphorylated Src indeed polarized in the migration direction (Fig. 1e).

We also examined the dynamics of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) distribution in migrating HL60 cells using the PH domain of Akt fused to green fluorescent protein (PHAkt–GFP). Again, PtdInsP_3 was polarized to the leading edge of differentiated HL60 cells migrating towards the cathode. Reversal of electric field polarity caused a rapid redistribution of PHAkt–GFP towards the direction of migration (Supplementary Fig. 6 and Movie 5). Cells treated with latrunculin still polarized PHAkt–GFP, suggesting that distribution of PtdInsP_3 to the leading edge in electrotaxis is independent of actin polymerization (Supplementary Fig. 7). These data show that electrotactic cues activate defined signalling pathways and induce polarization of PI(3)K and Src pathways to the leading edge of migratory cells.

To exclude a possible involvement of chemotactic effects in electric-field-directed cell migration, we used a set-up in which continual flow of culture medium perpendicular to the electrical vector was maintained throughout the experiment[23]; the directionality of electric-field-induced cell migration was again unaffected (Supplementary Fig. 8). Moreover, we used a mutant *Dictyostelium* strain that lacks the Gβ subunit and therefore does not have a chemotactic reponse[24]. Intriguingly, this mutant β$^-$ *Dictyostelium* strain still showed robust electrotactic responses (Supplementary Fig. 9 and Movie 6). Although the interplay between chemical and electrical gradients will be extremely relevant *in vivo*, these results indicate that electrical stimuli can act independently of local chemical gradients and chemokine sensing.

To provide genetic proof that these above signalling cascades are indeed important for electrotaxis, we examined the role of PI(3)Kγ in cells where its gene (phosphatidylinositol-3-kinase, catalytic, gamma subunit (*Pik3cg*); hereafter termed *p110γ*) was disrupted[25] (Supplementary Fig. 10). *p110γ*$^{-/-}$ cells showed impaired activation of Akt and partially reduced phosphorylation of Src, p38 and ERK in response to electric fields (Fig. 1d). Notably, loss of PI(3)Kγ markedly abrogated electrotactic migration of epithelial cells in single-cell migration assays and monolayer wound healing assays (Fig. 2; Supplementary Fig. 11 and Movies 7 and 8). In addition, electrotactic directionality was attenuated in primary cultures of neutrophils (Supplementary Fig. 12 and Movie 9) and dermal fibroblasts (Supplementary Fig. 13 and Movie 3) from *p110γ*$^{-/-}$ mice, confirming the importance of PI(3)Kγ signalling in electrotactic responses. To exclude the possibility that the electrotactic phenotypes in *p110γ*$^{-/-}$ cells were secondary to developmental alterations in these mutant mice, we blocked PI(3)K activity with wortmannin. Pharmacological

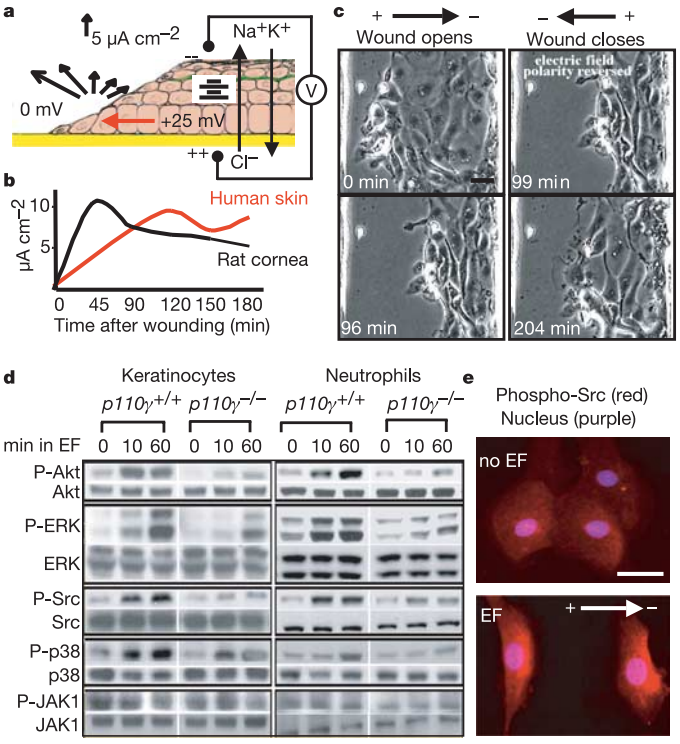

Figure 1 | Electrical signals direct cell migration in wound healing and activate selected signalling pathways. **a**, Wounding induces lateral electric fields directed towards the wound centre (red arrow), by collapsing the local transepithelial potential difference (V). Black arrows represent sizes and directions of currents. **b**, Directly measured currents increase over time in rat corneal and human skin wounds. **c**, **d**, An electric field (EF) directs migration of corneal epithelial cells in a monolayer model of wound healing (150 mV mm^{-1}; **c**) and activates Akt (Ser 473), Src (Tyr 416), ERK and p38 in primary cultures of mouse keratinocyte and mouse peritoneal neutrophils in serum-free medium (200 mV mm^{-1}; **d**). Disrupting *p110γ* attenuates activation of these signalling pathways. Phosphorylated JAK1 and JAK1 are shown as controls. **e**, Phosphorylated Src kinase polarizes in the direction of cell migration in electrotactic mouse keratinocytes (150 mV mm^{-1}). Scale bar, 20 μm.

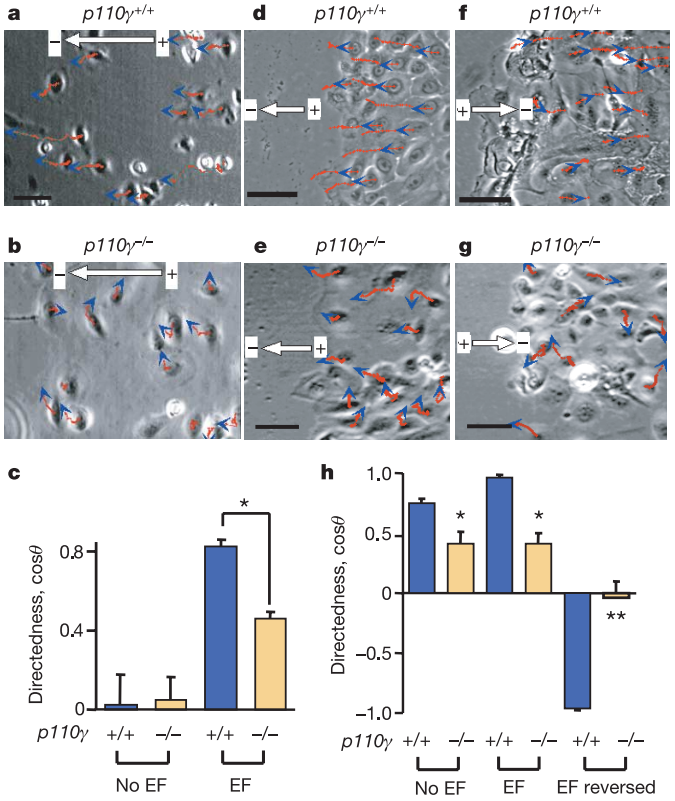

Figure 2 | Electrotaxis requires PI(3)Kγ. **a**–**c**, Impaired electrotactic responses in nonconfluent *p110γ*$^{-/-}$ keratinocytes. **d**–**h**, Attenuated electric field (EF)-directed migration of *p110γ*$^{-/-}$ keratinocytes in monolayer wound healing assays. Wild-type (*p110γ*$^{+/+}$) cells are shown as controls. Electric fields that are applied with polarity opposite to the default healing direction direct the cells to move away from the wound (**f**, **g**). Red lines and blue arrows represent trajectories and direction of cell movement. Data (mean ± s.e.m.) were quantified from at least four independent experiments (**c**, **h**). *$P < 0.05$; **$P < 0.01$, Student's *t*-test. Scale bars, 50 μm, EF = 200 mV mm^{-1}. See also Supplementary Movies 7 (for **a**–**c**) and 8 (for **d**–**h**).

inhibition of PI(3)K again inhibited keratinocyte migration in response to electrical signals (Supplementary Figs 14 and 15, and Movies 10 and 11). These data show that PI(3)Kγ controls electrotaxis and provide the first identification of a gene, *p110γ*, that controls electric-field-induced cell migration.

To verify that PI(3)Kγ regulates electrotaxis through $PtdInsP_3$ signalling, we investigated the effect of a tissue-specific deletion of the gene phosphatase and tensin homolog (*Pten*) in keratinocytes[26,27] (Fig. 3a). The lipid phosphatase PTEN negatively regulates the PI(3)K/Akt pathway by downregulating the amount of $PtdIns(3,4,5)P_3$. Genetic disruption of *Pten* markedly enhanced electric-signal-induced ERK and Akt phosphorylation (Fig. 3b). Notably, *Pten* deletion enhanced electric-field-directed keratinocyte migration (Fig. 3c, d; Supplementary Movie 12). Moreover, in monolayer wound healing assays, loss of *Pten* enhanced electric-field-directed keratinocyte migration both into the wound and away from the wound with significantly higher directedness ($P = 0.022$ and $P = 0.017$ respectively) and increased migration rates ($P = 0.027$ and $P = 0.024$ respectively) when compared with $Pten^{flox/flox}$ control keratinocytes (Fig. 3e–g; Supplementary Fig. 16 and Movie 13). These results indicate that PI(3)Kγ and the tumour suppressor PTEN mediate directional sensing of cell migration in response to electric signals.

Because our data were obtained from cell-culture experiments, we wanted to test whether this pathway is important for electrotactic wound healing after injury of a whole organ. We therefore tested whether PI(3)Kγ signalling is important for the healing of stratified epithelium in cornea explant wounds[12]. The direction and the magnitude of the wound electrical currents of cornea and skin from $p110\gamma^{-/-}$ mice were similar to those from wild-type mice. In wild-type tissue, the wound edge of the corneal stratified epithelium moved into the wound, and this movement was significantly ($P = 0.027$) enhanced by an electric field applied with the cathode directed into the wound (Fig. 4a, b). Genetic disruption of *p110γ* markedly impaired directed epithelial cell movement in response to electrical signals (Fig. 4c, d, g; Supplementary Movie 14). Application of an electric field with the polarity opposite to the default healing direction guided the stratified epithelial cells to migrate away from the wound, resulting in the wound opening up (Fig. 4e). Genetic disruption of *p110γ* abolished this response (Fig. 4f, g; Supplementary Movie 14). Thus, PI(3)Kγ expression is crucial for electrotaxis-regulated wound healing of a whole tissue.

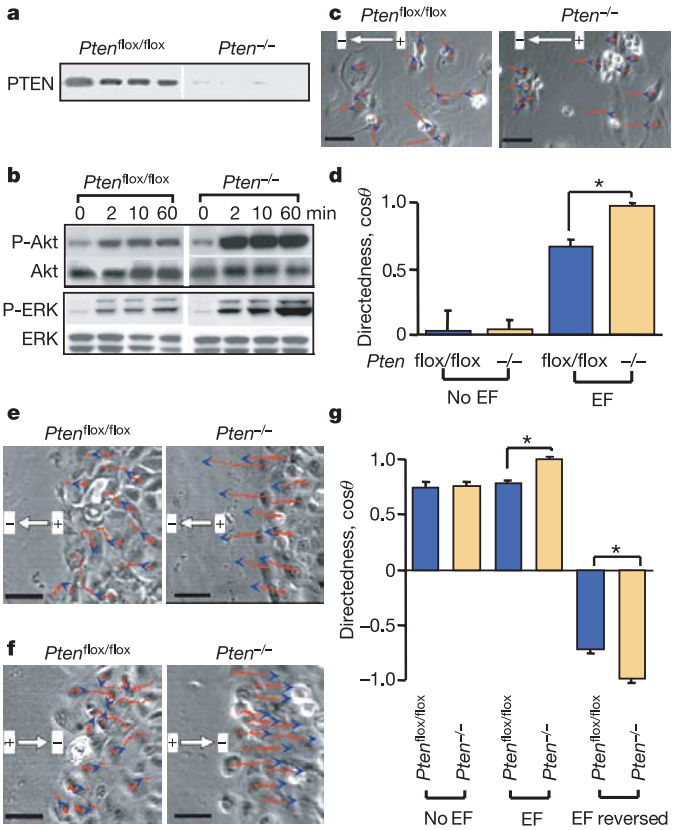

Figure 3 | The tumour suppressor PTEN negatively regulates electrotaxis. **a**, PTEN protein expression in keratinocytes. Four different cultures for each genotype are shown. **b**, Loss of PTEN expression in keratinocytes results in enhanced electric field (EF)-induced activation of Akt and ERK. **c**, **d**, Increased electrotactic migration of nonconfluent *Pten*-deficient keratinocytes. **e**–**g**, Loss of PTEN increases migration of keratinocytes in monolayer wound healing experiments in response to electric fields directed into (**e**, **g**) or away from (**f**, **g**) the wound. Red lines and blue arrows represent trajectories and direction of cell movement, respectively. Data are representative of at least four independent experiments with similar results. Quantification data are the mean ± s.e.m. (**d**, **g**). Scale bars, 50 μm, EF = 200 mV mm^{-1}. *$P < 0.05$, Student's *t*-test. See also Supplementary Movies 12 (for **c**, **d**) and 13 (for **e**–**g**).

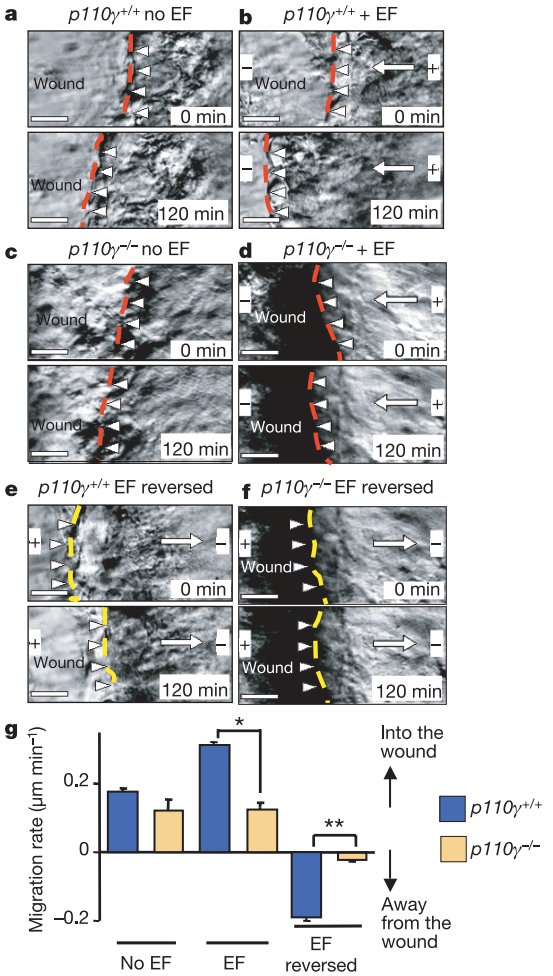

Figure 4 | PI(3)Kγ is required for electrotactic cell movement in wound healing of stratified epithelium in *ex vivo* cornea cultures. **a**, Stratified corneal epithelium migrate *in situ* to heal a wound (towards the left). **b**, This wound healing response is significantly enhanced by an electric field with the cathode at the wound. **c**, **d**, Impaired electric-field-mediated wound healing in corneas isolated from $p110\gamma^{-/-}$ mice. **e**, Electric fields applied with polarity opposite to the default healing direction direct the wound edge to migrate away from the wound. **f**, This response is impaired when *p110γ* is disrupted. Results were confirmed in three or more independent experiments for each response. **g**, Quantification of the migration rates of the healing cornea epithelium from 3–7 experiments for a period of 120 min at each condition. Data are the mean ± s.e.m. EF = 150 mV mm^{-1}. Scale bars, 50 μm. *$P < 0.05$, **$P < 0.01$, Student's *t*-test. See also Supplementary Movie 14.

Because all cell types and intracellular organelles maintain transmembrane electrical potentials owing to asymmetric ion transport, wounding results in strong and directional ion flow after disruption of epithelial cell layers[1,5–7]. To identify possible mediators that couple electric stimuli to intracellular responses, we tested the role of ion transporters in the electrotactic response. In particular, the Na^+/H^+ exchanger 1 (NHE1) has been implicated in directional cell migration[28]. Two different types of NHE1 inhibitor, cariporide and EIPA[29], abrogated electric-field-induced Akt activation (Supplementary Fig. 17a) and decreased the directedness of cell migration in electric fields (Supplementary Fig. 17b). These results suggest that directional Na^+/H^+ transport by the NHE1 ion exchanger might relay the electric signal to PI(3)K activation with subsequent directional migration. In addition to Na^+/H^+ exchangers, it is likely that other ion channels such as Cl^- channels (Supplementary Fig. 5) are also involved in electrotactic cell migration.

Although wound-induced electric currents have been known for more than 150 yr, the role of electrical signals in wound healing has long been discounted[1–3,9]. Moreover, such responses have not been confirmed genetically. Using multiple model systems, we have shown that electric currents can act as directional cues in cell movement and wound healing. These cues activate signalling pathways similar to those reported for chemotaxis[14–21]. Mechanistically, electric fields couple to directed cell migration through PI(3)Kγ and PTEN signalling. These experiments identify the first genes that modulate cell movements and wound healing in response to electrical currents.

METHODS

See Supplementary Information for full details.

Mutant mice, cell and tissue culture, and wound healing assays. *Pten*^{flox} and *p110*γ mutant mice and the β$^-$ mutant *Dictyostelium* strain have been described[24–27]. Primary cultures of keratinocytes from *Pten*$^{flox/flox}$ mice were treated with adenovirus carrying GFP and Cre to delete the floxed *Pten* allele. In all experiments, littermate controls were used. Wound healing in corneal explant organ cultures and *in vivo* were done as described[7,12]. All animal experiments were performed in accordance with institutional guidelines.

Electric fields. Endogenous wound electric currents were measured with a vibrating probe system[5]. DC electric fields of indicated strengths were applied in electrotactic chambers with modification for use in organ culture[30]. Directedness was used as a parameter to define how cells migrate directionally in response to electric fields. Directedness values approaching one indicate migration directionally in the electric fields, whereas directedness values of or close to zero indicate random migration.

Western blot, immunofluorescene and time-lapse imaging. Primary cultures of keratinocytes and neutrophils, and wild-type fibroblasts were starved in serum-free medium before electric field stimulation. Cells were lysed and samples were probed with appropriate antibodies. For immunofluorescence microscopy, keratinocytes were exposed to electric fields, fixed, permeabilized for antibody labelling, and mounted in medium containing 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). Dimethyl-sulphoxide-differentiated HL60 cells expressing PHAkt–GFP[19] were exposed to an electric field. All time-lapse video images were recorded and analysed with a MetaMorph system.

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1. Barker, A. T., Jaffe, L. F. & Vanable, J. W. Jr The glabrous epidermis of cavies contains a powerful battery. *Am. J. Physiol.* **242,** R358–R366 (1982).
2. Foulds, I. S. & Barker, A. T. Human skin battery potentials and their possible role in wound healing. *Br. J. Dermatol.* **109,** 515–522 (1983).
3. McCaig, C. D., Rajnicek, A. M., Song, B. & Zhao, M. Controlling cell behaviour electrically: current views and future potential. *Physiol. Rev.* **85,** 943–978 (2005).
4. DuBois-Reymond, E. Vorläufiger Abriss einer Untersuchung uber den sogenannten Froschstrom und die electomotorischen Fische. *Ann. Phy. U. Chem.* **58,** 1–30 (1843).
5. Jaffe, L. F. & Nuccitelli, R. An ultrasensitive vibrating probe for measuring steady extracellular currents. *J. Cell Biol.* **63,** 614–628 (1974).
6. Borgens, R. B., Vanable, J. W. Jr & Jaffe, L. F. Bioelectricity and regeneration: large currents leave the stumps of regenerating newt limbs. *Proc. Natl Acad. Sci. USA* **74,** 4528–4532 (1977).
7. Reid, B., Song, B., McCaig, C. D. & Zhao, M. Wound healing in rat cornea: the role of electric currents. *FASEB J.* **19,** 379–386 (2005).
8. Keese, C. R., Wegener, J., Walker, S. R. & Giaever, I. Electrical wound-healing assay for cells *in vitro*. *Proc. Natl Acad. Sci. USA* **101,** 1554–1559 (2004).
9. Robinson, K. R. & Messerli, M. A. Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion. *BioEssays* **25,** 759–766 (2003).
10. Singer, A. J. & Clark, R. A. Cutaneous wound healing. *N. Engl. J. Med.* **341,** 738–746 (1999).
11. Chiang, M., Robinson, K. R. & Vanable, J. W. Jr Electrical fields in the vicinity of epithelial wounds in the isolated bovine eye. *Exp. Eye Res.* **54,** 999–1003 (1992).
12. Zhao, M., Song, B., Pu, J., Forrester, J. V. & McCaig, C. D. Direct visualization of a stratified epithelium reveals that wounds heal by unified sliding of cell sheets. *FASEB J.* **17**, 397–406 (2003).
13. Song, B., Zhao, M., Forrester, J. & McCaig, C. Nerve regeneration and wound healing are stimulated and directed by an endogenous electrical field *in vivo*. *J Cell Sci.* **117,** 4681–4690 (2004).
14. Song, B., Zhao, M., Forrester, J. V. & McCaig, C. D. Electrical cues regulate the orientation and frequency of cell division and the rate of wound healing *in vivo*. *Proc. Natl Acad. Sci. USA* **99,** 13577–13582 (2002).
15. Devreotes, P. & Janetopoulos, C. Eukaryotic chemotaxis: distinctions between directional sensing and polarization. *J. Biol. Chem.* **278,** 20445–20448 (2003).
16. Rickert, P., Weiner, O. D., Wang, F., Bourne, H. R. & Servant, G. Leukocytes navigate by compass: roles of PI3Kγ and its lipid products. *Trends Cell Biol.* **10,** 466–473 (2000).
17. Kimmel, A. R. & Parent, C. A. The signal to move: *D. discoideum* go orienteering. *Science* **300,** 1525–1527 (2003).
18. Firtel, R. A. & Chung, C. Y. The molecular genetics of chemotaxis: sensing and responding to chemoattractant gradients. *BioEssays* **22,** 603–615 (2000).
19. Servant, G. *et al.* Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. *Science* **287,** 1037–1040 (2000).
20. Xu, J. *et al.* Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* **114,** 201–214 (2003).
21. Iijima, M. & Devreotes, P. Tumor suppressor PTEN mediates sensing of chemoattractant gradients. *Cell* **109,** 599–610 (2002).
22. Funamoto, S., Meili, R., Lee, S., Parry, L. & Firtel, R. A. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109,** 611–623 (2002).
23. Erickson, C. A. & Nuccitelli, R. Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *J. Cell Biol.* **98,** 296–307 (1984).
24. Wu, L., Valkema, R., Van Haastert, P. J. & Devreotes, P. N. The G protein β subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. *J. Cell Biol.* **129,** 1667–1675 (1995).
25. Sasaki, T. *et al.* Function of PI3Kγ in thymocyte development, T cell activation, and neutrophil migration. *Science* **287,** 1040–1046 (2000).
26. Suzuki, A. *et al.* T cell-specific loss of Pten leads to defects in central and peripheral tolerance. *Immunity* **14,** 523–534 (2001).
27. Suzuki, A., Sasaki, T., Mak, T. W. & Nakano, T. Functional analysis of the tumour suppressor gene PTEN in murine B cells and keratinocytes. *Biochem. Soc. Trans.* **32,** 362–365 (2004).
28. Denker, S. P. & Barber, D. L. Cell migration requires both ion translocation and cytoskeletal anchoring by the Na-H exchanger NHE1. *J. Cell. Biol.* **159,** 1087–1096 (2002).
29. Masereel, B., Pochet, L. & Laeckmann, D. An overview of inhibitors of Na^+/H^+ exchanger. *Eur. J. Med. Chem.* **38,** 547–554 (2003).
30. Zhao, M., Agius-Fernandez, A., Forrester, J. V. & McCaig, C. D. Orientation and directed migration of cultured corneal epithelial cells in small electric fields are serum dependent. *J. Cell Sci.* **109,** 1405–1414 (1996).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions M.Z. designed the experiments, took part in the cell migration and western blotting experiments, analysed the results and wrote the paper. J.M.P. designed the genetic analysis of the electric-field-induced signalling pathway, analysed the data and wrote the paper. B.S. did the *in vivo* experiments, most experiments with cells and tissues from transgenic mice. B.S. and J.P. did most of the cell migration and wound healing assays. T.W. performed the first signalling experiment and genotyping. B.R. performed the vibrating probe measurements. G.T., F.W. and P.W. did the experiments with HL60 cells. B.S., A.G. and Y.G. did the experiments on fibroblasts. P.N.D., A.S. and T.S. provided mouse and *D. discoideum* lines essential for the experiments. J.V.F., H.B. and C.D.M. helped with some of the experimental design, writing and analysis of the data. All authors discussed the results and commented on the manuscript.

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