Integrin α6β4 and TRPV1 channel coordinately regulate directional keratinocyte migration.

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Abstract

The directional migration of epithelial cells is crucial for wound healing. Among integrins, a family of cell adhesion receptors, integrin β4 has been assumed to be a promigratory factor, in addition to its role in stable adhesion. In turn, Ca^{2+} signaling is also a key coordinator of migration. Keratinocytes reportedly express transient receptor potential vanilloid channels (TRPV1); however, the function of these channels as a regulator of intracellular Ca^{2+} level in cell migration has remained uncharacterized. In the present study, we investigated the role of TRPV1 in directional migration related to integrin β4 using a scratch wound assay on a confluent monolayer sheet of murine keratinocytes (Pam212 cells). Double immunofluorescence staining revealed the *de novo* expression of integrin β4 and TRPV1 in migrating cells at the wound edge in response to scratch wounding, and both expression levels were almost matched. Epidermal growth factor (EGF) not only promoted keratinocyte migration, but also caused the further up-regulation of both integrin β4 and TRPV1. In addition, the knockdown of the integrin β4 or TRPV1 gene significantly impeded wound closure. The TRPV1 agonist capsaicin significantly promoted migration, while a selective TRPV1 antagonist inhibited it. The gene knockdown of TRPV1 inhibited the expression of the integrin β4 gene and that of β4 protein in migrating cells. These findings suggest that TRPV1 may stimulate directional migration directly by eliciting a Ca^{2+} signal or indirectly via integrin β4 expression.

1. Introduction

Cell migration has been shown to be an important process that mediates wound healing. Cells are thought to migrate in a directed manner in response to various extracellular environmental cues, such as chemotaxis [1]. During directional migration, leader cells near the wound edge respond to environmental cues, while follower cells located behind the leader cells migrate based on cues from their neighboring cells [2].

The molecular mechanism responsible for the coordination of spatially organized cell migration has not been fully clarified. Integrin α6β4 (hereafter referred to as integrin β4 since β4 pairs only with α6) is primarily expressed in epithelial cells and is a component of hemidesmosomes (HD), which anchor epithelial cells to the basement membrane [3,4]. The idea that integrin β4 may play an active role in the migration of keratinocytes and carcinoma cells has recently gained acceptance [5,6]. Integrin β4 has an unusual cytoplasmic region consisting of 1088 amino acids residues, which is much longer than those of other β subunits [7], and it associates with the intermediate filament system through other intracellular HD components [8]. When migration occurs in response to wounding, epithelial cells are thought to shed HD, thereby reducing their tight attachment to the extracellular matrix via integrin β4. In addition, studies have suggested that the cytoplasmic region of integrin β4 might be important in signaling associated with various cellular functions, such as migration in a manner that is independent of the function of integrin β4 as an adhesion receptor [9,10]. On the other hand, integrin β4 reportedly appeared in a restricted and temporal manner in basal and suprabasal cells that had recently migrated from the edge of a wounded site during *in vivo* experiments [11], suggesting it has a promigratory role. Despite this evidence and because this evidence was not thought to be related to migration, but rather to the anchoring function of integrin β4 involving HD, little attention has been directed to

such phenomena as they relate to migration or to the mechanisms that regulate such patterns of integrin β4 expression. Further confirmation of whether integrin β4 exhibits a promigratory function and how this effect may arise is needed.

Ca^{2+} signals are known to coordinate cell migration, with cell migration relying on the precise temporal and spatial regulation of intracellular Ca^{2+} ($[Ca^{2+}]i$) concentrations. Besides spinal and peripheral nerves, epithelial cells, such as keratinocytes, have been reported to express transient receptor potential vanilloid channels TRPV1 [12]. Accumulating evidence has suggested that TRPV1 play noticeable roles in the regulation of the $[Ca^{2+}]i$ level in both excitable and nonexcitable cells. As for the epidermal function of TRPV1, most discussion has focused on their function as a sensor for noxious stimuli in the skin [13], in addition to their critical role in maintaining the epidermal barrier function of the body [14]. TRPV1 has recently been suggested to play a novel role in cell motility via $[Ca^{2+}]i$ activity, which was affected by these channels in a single-cell migration study [15]. Furthermore, the impairment of corneal epithelial wound healing in a TRPV1-deficient mouse has been reported [16]. However the role of TRPV1 in directional cell migration and the mechanism involved remains unclear.

Using a scratch wound assay, we found that integrin β4 was densely expressed in concert with the abundant expression of TRPV1 in leader cells at the free edge during *in vitro* directional migration, and this expression pattern was related to the pro-migratory action. We postulated the existence of a novel mechanism for regulating keratinocyte migration via interactions between integrin β4 and Ca^{2+} influx through TRPV1 channels.

2. Materials and methods

2.1. Materials

Capsaicin (Sigma-Aldrich, St. Louis, MO), TRPV1 antagonist AMG9810 (Sigma-Aldrich), mouse natural EGF (Corning Life Sciences, Middlesex, MA), and EGFR kinase inhibitor tyrphostin AG1478 (Cell Signaling Technology, Danvers, MA) were used. Each stock solution of capsaicin (20 mM), AMG9810 (10 mM), or AG1478 (1 mM) was created by dissolving in DMSO and was then used after dilution with the culture medium.

2.2. Cell culture

Pam212, a mouse keratinocyte cell line, was kindly gifted by Dr. Yuspa (Center for Cancer Research, National Cancer Institute, MD, USA) [17]. These cells were cultured in Joklik modified MEM (0.05 mM $CaCl_2$) with antibiotics containing 10% heat-inactivated FBS. The cultures were performed in a humidified atmosphere with 5% CO_2 at 37˚C.

2.3. Cell migration assay

Keratinocytes were plated as monolayers of 3.5 x 10^6 cells per well on 24-well plates. When the cells become confluent, a cell-free band with a width of approximately 500 μm was generated at the center of each well uniformly using a 200-μL pipet tip. Wounded cell layers were washed twice with PBS and then incubated with growth medium containing 1 μg/mL of mitomycin C to inhibit cell proliferation. Wound closure was captured using an optical microscope (TMS) with a digital camera (DS-Fi1) (Nikon, Japan) at 0 and 12 h (Fig. S1), and the migration distance of the leading edge of the monolayer was estimated using ImageJ software (NIH, MD, USA).

2.4. Gene knockdown by siRNA

siRNA transfection was performed using Lipofectamine® RNAiMAX (Invitrogen, UK),

following the manufacturer's reverse transfection protocol in a 24-well plate format. To each well containing the siRNA duplex (12 pmol)-reagent complexes, 500 μL of diluted cells in complete growth medium without antibiotics were added. We designed the target-specific siRNA duplexes of integrin β4 and TRPV1 using online siRNA design software with a minimized off-target effect (Enhanced siDirect [https://sidirect.jp/esd/]). The following siRNAs targeting integrin β4 (NM_001005608.2) were used: siRNA#1, sense 5'-GUAAACAUCACCAUUAUCAAG-3' and antisense 5'-UGAUAAUGGUGA UGUUUACCA-3'; and siRNA#2, sense 5'-CUUUUGUUCUGCACUUAAUAA-3' and antisense 5'-AUUAAGUGCAGAACAAAAGGC-3'. The following siRNAs targeting TRPV1 (NM_001001445.1) were also used: siRNA#1, sense 5'-GGACGCAAGCACUCG AGAUAG-3' and antisense 5'-AUCUCGAGUGCUUGCGUCCCU-3'; and siRNA#2, sense 5'-GCACUAUACGGGAUCCCUUAA-3' and antisense 5'-AAGGGAUCCCGU AUAGUGCUU-3'. The cell populations were transfected with a pool of the two siRNAs (siRNA #1 + siRNA #2), all targeting the same gene.

2.5. Immunocytochemistry

Keratinocytes were cultured on type I-collagen-coated LabTek II Chambers (Nalgen Nunc, IL, USA). As mentioned above, immunostaining was performed 6 hours after the generation of the wounded-cell-layers. Briefly, after washing with PBS, the cells were fixed in 4% paraformaldehyde-PBS for 20 min and then permeabilized with 0.1% Triton-X100-PBS. After blocking with 10% goat or rabbit serum-PBS, the cells were incubated with the primary antibodies overnight at 4°C, followed by incubation with the secondary antibodies for 1 h. The primary antibodies used were as follows: goat anti-TRPV1 antibody (sc-12498, diluted 1:100) and mouse anti-integrin β4 antibody (sc-55514, diluted 1:100) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA); and hamster anti-integrin β1 antibody (HMβ1-1, diluted 1:200; BioLegend, San Diego,

CA, USA). Alexa Fluor 488-conjugated anti-goat for TRPV1, Alexa Fluor 488- or 568-conjugated anti-mouse for integrin β4, and Alexa Fluor 647-conjugated anti-hamster for integrin β1 (each diluted 1:1000) (Molecular Probes, Eugene, OR, USA) were used as the secondary antibodies. Alexa Fluor 555-phalloidin (diluted 1:20) (Molecular Probes) was used for actin staining. The cells were also counterstained with the nuclear stain DAPI.

2.6. Image analysis

All the images were obtained using multiple label immunofluorescence and confocal laser scanning microscopy (model LSM710) and were processed using the LSM software ZEN 2009 (both from Carl Zeiss, Jena, Germany). Data collection using z-stack data acquisition software was performed typically in stacks of 12 slices. The 3D images constructed using confocal z-stacks in the maximum intensity projection mode were displayed as a 2D image and enabled a higher accuracy than single 2D images when visualizing the expression levels of distinct molecules with peak expression strengths at different cell depths (i.e., found in different z-sections). For the semi-quantitative analysis, the stack of images for the TRPV1 channel or for integrin β4 was analyzed using the 3D-Object Counter plugin for ImageJ [18].

2.7. Real-time PCR

The quantitative PCR was performed using the One-step SYBR PrimeScript PLUS RT-PCR kit (Takara BIO, Otsu, Japan). Fifty picograms of total RNA per 25 μL of the reaction volume was used for each sample, and the samples were examined in duplicate. The following primers used for the SYBR Green assay were purchased from TaKaRa Bio (Primer set ID): MA144661 for TRPV1, MA129318 for integrin β1, MA067708 for integrin β4, and MA031262 for Hprt. The comparative $\Delta\Delta C^T$ method was used to

calculate the expression of the target gene normalized to an endogenous control (Hprt).

2.8. Cobalt histochemistry

Cobalt histochemistry was performed using a slight modification of a method described by Pecze [19]. Six hours after wound scratching, the keratinocytes that were attached to the coverslips were washed twice with buffer A (NaCl, 57.5 mM; KCl, 5 mM; $MgCl_2$, 2 mM; HEPES, 10 mM; glucose, 12 mM; sucrose, 139 mM; pH7.4). The cells were then incubated at 37°C in cobalt-uptake solution (buffer A + 5 mM $CoCl_2$) containing 1 μM capsaicin for 10 min. Following washing with buffer A, the water-soluble cobalt taken up by the cells was precipitated using a 0.2% ammonium sulfide solution in buffer A for 1 min, resulting in the formation of dark, water-insoluble CoS within their interiors of TRPV1-positive cells.

Statistical analysis

Different values among the experimental groups were analyzed using a one-way ANOVA and the post-hoc Scheffe test, with a value of $P < 0.05$ indicating statistically significant differences. The results were presented as the means ± SEM.

3. Results and discussion

3.1. *De novo* expression of integrin β4 in keratinocytes located near the wound edge

To characterize involvement of integrin β4 in the directional migration of keratinocytes, immunofluorescence staining was performed after wound scratching in a confluent monolayer of Pam212 cells. For comparison purposes, the expression of integrin β1, which is also thought to be related to directional migration, was also investigated. In Figure 1a, the 3D-reconstructed images showed the restriction of

intensive integrin β4 immunoreactivity to a relatively loose cell cluster at the free edge; in these cell clusters, the actin bundles were indistinct and a motile cell shape (elongated/spindle-shaped/raised) was visible. Furthermore, the nuclear shrinkage and higher DAPI fluorescence intensities (showing chromatin condensation), both of which characterize migrating cells [20], were observed in these cells (red arrows in Fig. 1a). On the other hand, the expression of integrin β1 was prominent in the posterior inner cells, which maintained an apparent actin filament structure and exhibited a flat, cobblestone-like cell shape, indicating a higher adhesiveness. Many reports have revealed that integrin β4 has promigratory functions in epithelial and carcinoma cells, and these functions do not always depend on the adhesive function of this integrin [5]. Reportedly, the overexpression of an integrin β4 clone enhanced the chemotactic migration of MDA-MB-435 cells [21]. Rezniczek et al. [22] showed that the ectopic overexpression of integrin β4 cytoplasmic domains caused the *in vitro* self-interaction of this domain, together with the disruption of intermediate filament network to HD. Additionally, they reported that cells overexpressing integrin β4 frequently have a rounder morphology, which was apparent during the process of detachment from the substrate and was also observed in a second cell layer in dense cultures. The intensive expression of integrin β4 observed in the leading cells (Fig. 1) may lead to the above-mentioned self-interaction of integrin β4 cytoplasmic domains; as a result, these cells may be able to move easily after the disruption of the HD and the changes in morphology, possibly explaining the up-regulation of integrin β4 in motile cells at the leading front.

3.2. *De novo* expression of TRPV1 in keratinocytes located near the wound edge

Here, we studied the role of TRPV1 in keratinocyte migration during wound healing using immunofluorescence staining. Since it has been shown that intensive

integrin β4 is localized at the wound edge (Fig. 1a), we also performed double labeling for integrin β4 and TRPV1 (Fig. 1b.) We found that the dense immunoreactivity of TRPV1 was restricted to cells located at the wound edge, similar to the abundant and localized expression of integrin β4.

3.3 EGF-induced enhancement of the keratinocyte migration and the expression of TRPV1 and integrin β4 near the wound edge

Keratinocyte chemotaxis induced by ligands for epidermal growth factor receptor (EGFR) has been well recognized. Upon wounding, EGFR ligands released from epithelial cells work in an autocrine manner to stimulate cell migration both in vivo and *in vitro* [9, 23]. We next assessed the influence of stimulation by EGF on the cell migratory response and the expression of TRPV1 and integrin β4 during migration. EGF (10 ng/mL) administered immediately after scratching significantly promoted the migration of keratinocytes (Fig. 2a). On the other hand, a specific inhibitor of EGFR tyrosine kinase, AG1478 (1 μM), even without EGF, inhibited the migration remarkably (Fig. 2b), suggesting that autocrine signaling induced by EGF may be involved in this migration assay.

Immunocytochemistry showed that EGF caused an increase in the number of cells expressing integrin β4, as well as the number expressing TRPV1 (Fig. 2c). A quantitative examination revealed that the percentage of cells with a dense expression of TRPV1 and integrin β4 near the wound edge increased significantly in response to EGF treatment (Fig. 2d). Furthermore, the inhibition of EGFR using AG1478 reduced the expressions of both of the molecules (Fig. 2e and S2). Since the up-regulation of both integrin β4 and TRPV1 coincides with the promigratory effects of exogenous EGF and the down-regulation of these molecules concurs with the antimigratory effects of AG1478, integrin β4 and TRPV1 may coordinately regulate directional migration when

EGF signaling is triggered by scratch wounding.

3.4 Cobalt influx induced by scratch wounding and capsaicin, and effect of capsaicin on keratinocyte migration

Co^{2+}-uptake is a surrogate indicator for Ca^{2+} influx, and its induction by capsaicin has been routinely used to identify functional TRPV1 [24]. Co^{2+} uptake was much higher in scratch wounded cells (Fig. 3a) compared to intact cells (Fig. 3b). Co^{2+} uptake was highest in migrating cells near the wound edge, which was similar to the pattern of TRPV1 immunoreactivity (Fig 1b).

Capsaicin, a potent TRPV1 agonist, strongly increased Co^{2+} uptake in scratched (Fig. 3c) and to a lesser extent in intact cells (Fig. 3d). Capsaicin also increased cell migration which can be inhibited with AMG9810 (5 μM), a TRPV1 antagonist (Fig. 3e). Altogether, the evidence suggests that TRPV1 activity promotes cell migration. In whole-cell voltage clamp bioassays using mouse keratinocytes, even 10 μM of capsaicin (i.e., EC_{50} = 0.3 μM in dorsal root ganglia) failed to evoke any detectable currents [25]. This evidence agrees with that obtained using a cell sheet without wound scratching, in which the cobalt uptake induced by capsaicin was limited to several cells (Fig. 3d). Thus, keratinocytes ordinarily express a few TRPV1 channels, and upon scratching, the *de novo* expression of TRPV1 occurs in a region restricted to the leader cells in a row facing the wound space, suggesting that this expression pattern may contribute to directional migration (Figs. 1b and 3a).

The capsaicin dosage used here is consistent with the dose (1 μM or less) that is ordinarily used for the activation of native or cloned TRPV1. Contradictory data has suggested that capsaicin may impair wound healing (at a higher dose of over 100 μM) [26] or that it may improve wound healing (at lower dose) [27]. Our results support the latter conclusion and may provide a clue to the solution of this discrepancy.

Additionally, AMG9810 used alone significantly inhibited the migration. AMG9810 has also been shown to compete with TRPV1 activation induced by endogenous ligands, known as endovanilloids. The epidermal layer of the skin is a site of active arachidonic acid metabolism, and the main product of keratinocytes is 12-HETE (proposed as a possible endovanilloid), which induces the chemotaxis of keratinocytes in wound healing [28], suggesting that its biological effect might be elicited via TRPV1 activation. Furthermore, subsequent to tissue damage, TRPV1 activation can reportedly occur in response to the damage-mediated release of the endogenous agonists [29]. These evidences and our results suggest that the endogenous activation of TRPV1 accompanying Ca^{2+} influx likely occurs during keratinocyte migration.

3.5. TRPV1 gene silencing causes suppression of integrin β4 expression

Next, gene knockdown experiments were performed to confirm the role of integrin β4 and TRPV1 on scratch-induced directional migration and to investigate whether any relationship exists between these two molecules. When the knockdown efficiency of each siRNA was first observed using cell cultures without scratching, integrin β4 siRNA effectively decreased only the gene expression of β4 itself, while TRPV1 siRNA led to not only the gene silencing of TRPV1, but also that of integrin β4 (Fig. 4a). Since the gene expression of integrin β1 was unchanged under the experimental conditions (data not shown), TRPV1 gene silencing might conceivably cause the selective down-regulation of the integrin β4 gene. A migration assay was then performed at 24 hours after the reverse transfection of siRNA. Keratinocyte migration was significantly suppressed by the siRNA of integrin β4 or TRPV1, although the inhibition induced by integrin β4 siRNA was weaker than that induced by TRPV1 knockdown (Fig. 4b). As expected, silencing of TRPV1 also down-regulated integrin β4 expression in scratch wounds (Fig. 4c, d). Furthermore, AMG9810 decreased the

expression of integrin β4 without the down-regulation of TRPV1 at the wound edge in squamous cell carcinoma HSC3 cells (Fig. S3), suggesting that Ca^{2+} entry through TRPV1 have an influence on the β4 expression. Reportedly, Ca^{2+} entry via TRPV1 triggers downstream pathways, leading to the activation of transcriptional factors such as NFAT and CREB and the initiation of gene expression [30, 31]. These findings suggest that the up-regulated Ca^{2+} signal via the increase in TRPV1 expression may positively regulate the expression of promigratory integrin β4 in migrating cells at the wound edge. However, further studies will be needed to determine the precise mechanisms.

In summary, we have presented the up-regulation of β4 integrin expression may be associated with migration in motile cells. In addition, the increase in Ca^{2+} influx via TRPV1 channels increases keratinocyte migration and β4 integrin expression. The inducible expression of TRPV1 and β4 integrin at the front edge of the wound may serve as a driving force for directional cell migration.

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Legends

Fig. 1 Expression of integrin β4 and integrin β1 during keratinocyte migration upon scratch wounding, as observed using immunofluorescence. Three-dimensional reconstructed images of the same field are shown for (a) integrin β4 (green), integrin β1 (purple), actin (red), and nuclei (blue). The large white upper rightward arrow indicates the direction of migration. A white background and shadow option was adopted to show the image clearly. The red arrows in (a) indicate nuclei with a dense DAPI intensity, indicating the migrating cells. (b) The double labeling of TRPV1 (green) and integrin β4 (red) in the same field is presented as 2D images. The white lines indicate the front line of the migration. Scale bar: 20 μm.

Fig. 2. EGF induced changes in TRPV1 and integrin β4 expression during cell migration. (a) Promotion of scratch-induced migration by EGF and (b) inhibition of migration by AG1478. The data represent means and SEM of 3 independent experiments with 6 wells per drug. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. (c) Increase in expression of TRPV1 (green) and integrin β4 (red) induced by EGF presented as 3D images of immunofluorescence. Scale bar: 20 μm. Semi-quantitative analysis of the effects of EGF (d) and AG1478 (e) using 3D images.

Fig. 3. (a-d) Cobalt accumulation induced by scratch wounding and capsaicin. Dark cobalt sulfide precipitation, as a marker of Ca^{2+} influx, is visible in the front-row cells migrating (the area surrounded by the dashed line) in (a) a control monolayer and (c) a capsaicin-treated monolayer with a scratch wound. (b) The control and (d) a capsaicin-treated monolayer without a scratch wound. Scale bar: 20 μm. (e) Effect of capsaicin (1 μM) on keratinocyte migration upon scratch wounding. The data represent means and SEM of 3 independent experiments with 6 wells per drug. $*P < 0.05$ and $**P$

< 0.01.

Fig. 4. Gene knockdown-induced changes in TRPV1 and integrin β4 expression during cell migration. (a) Knockdown efficiency in the expression of the TRPV1 gene (left) and the integrin β4 gene (right) by the corresponding siRNAs. The data represent means and SEM of 3 independent experiments with two wells per siRNA on six-well plates. (b) Effects of gene knockdown in cell migration upon scratch wounding. The data represent means and SEM of 3 independent experiments with 6 wells per siRNA. (c) Decrease in expression of TRPV1 (green) and integrin β4 (red) induced by TRPV1 gene knockdown as shown using 3D immunofluorescence images and (d) a semi-quantitative analysis based on the 3D images (left, TRPV1; right, integrin β4). Scale bar: 20 μm. *P< 0.05 and **P< 0.01.

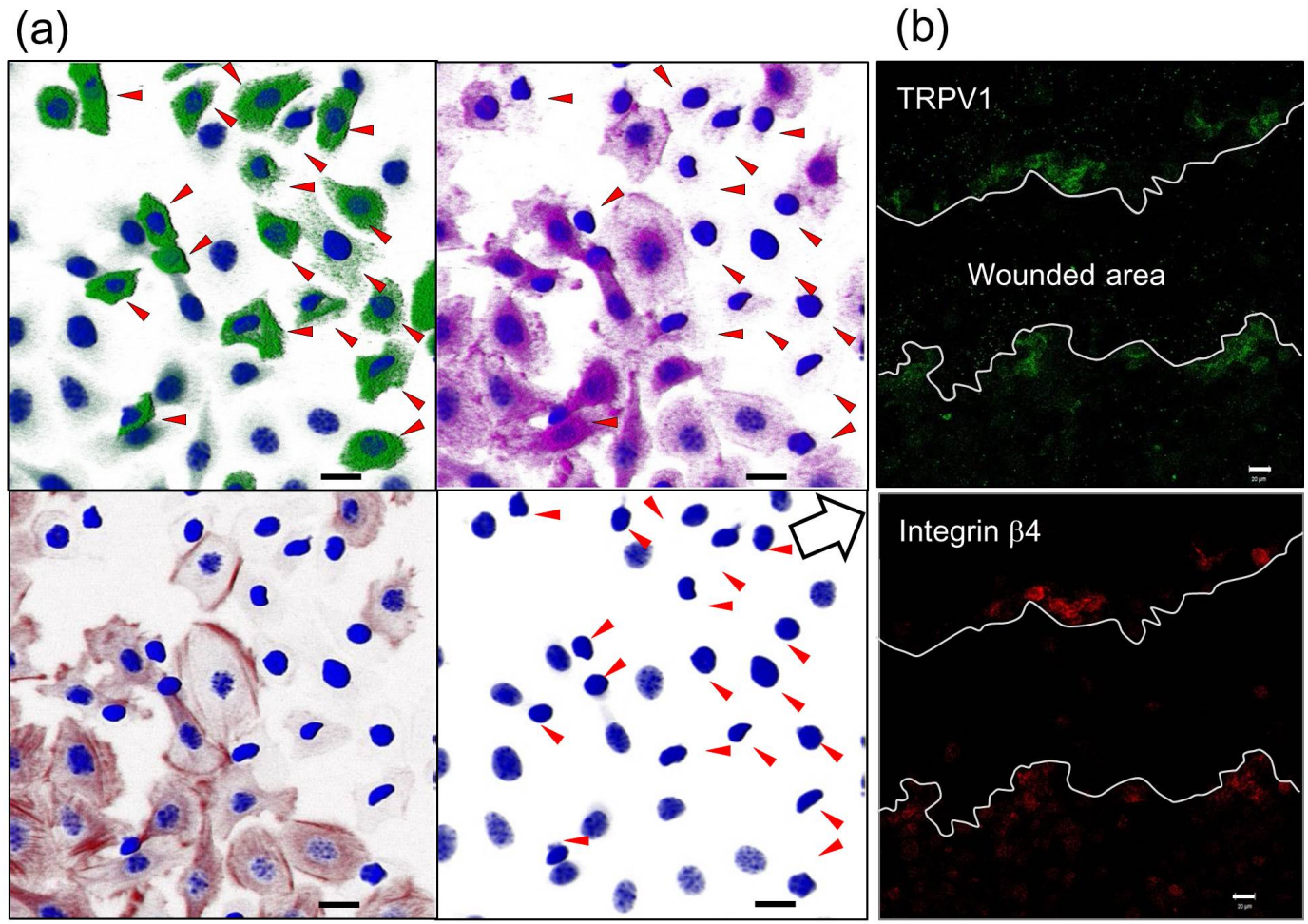
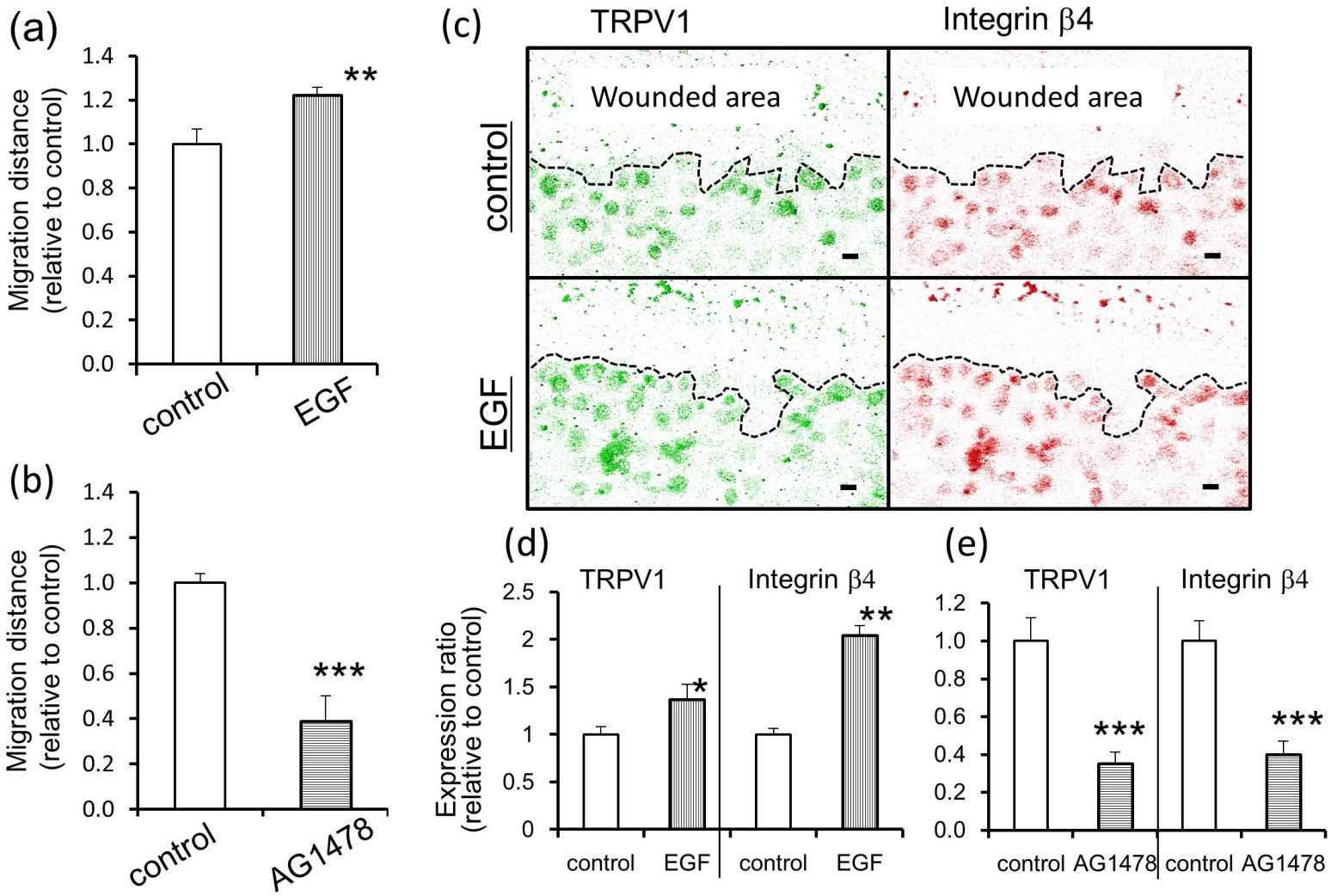


Fig. 1


(a)
Migration distance (relative to control)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
control
EGF
(b)
Migration distance (relative to control)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
control
AG1478
(c)
TRPV1
Integrin β4
Wounded area
Wounded area
control
EGF
(d)
TRPV1
Integrin β4
Expression ratio (relative to control)
2.5
2
1.5
1
0.5
0
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EGF
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AG1478

Fig. 2

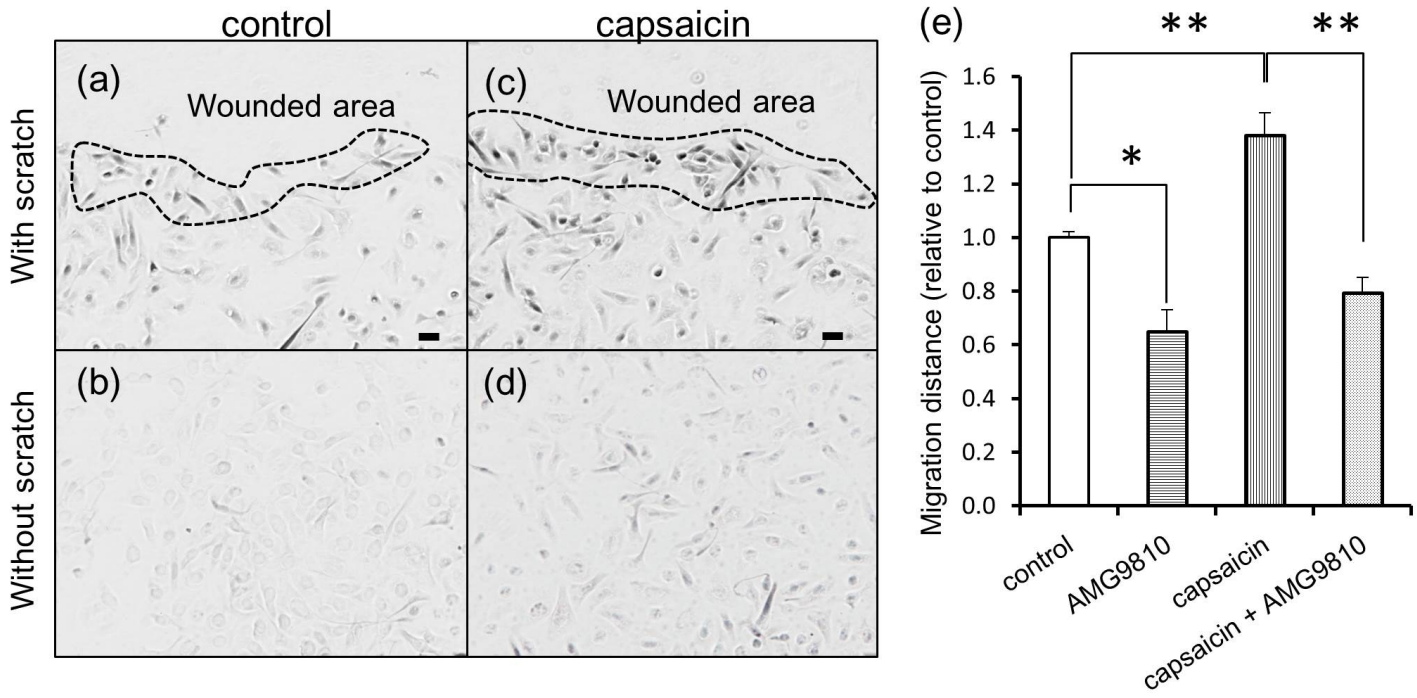
control
capsaicin
(a)
Wounded area
(c)
Wounded area
With scratch
Without scratch
(b)
(d)
(e)
**
**
*
Migration distance (relative to control)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
AMG9810
capsaicin
capsaicin + AMG9810

Fig. 3

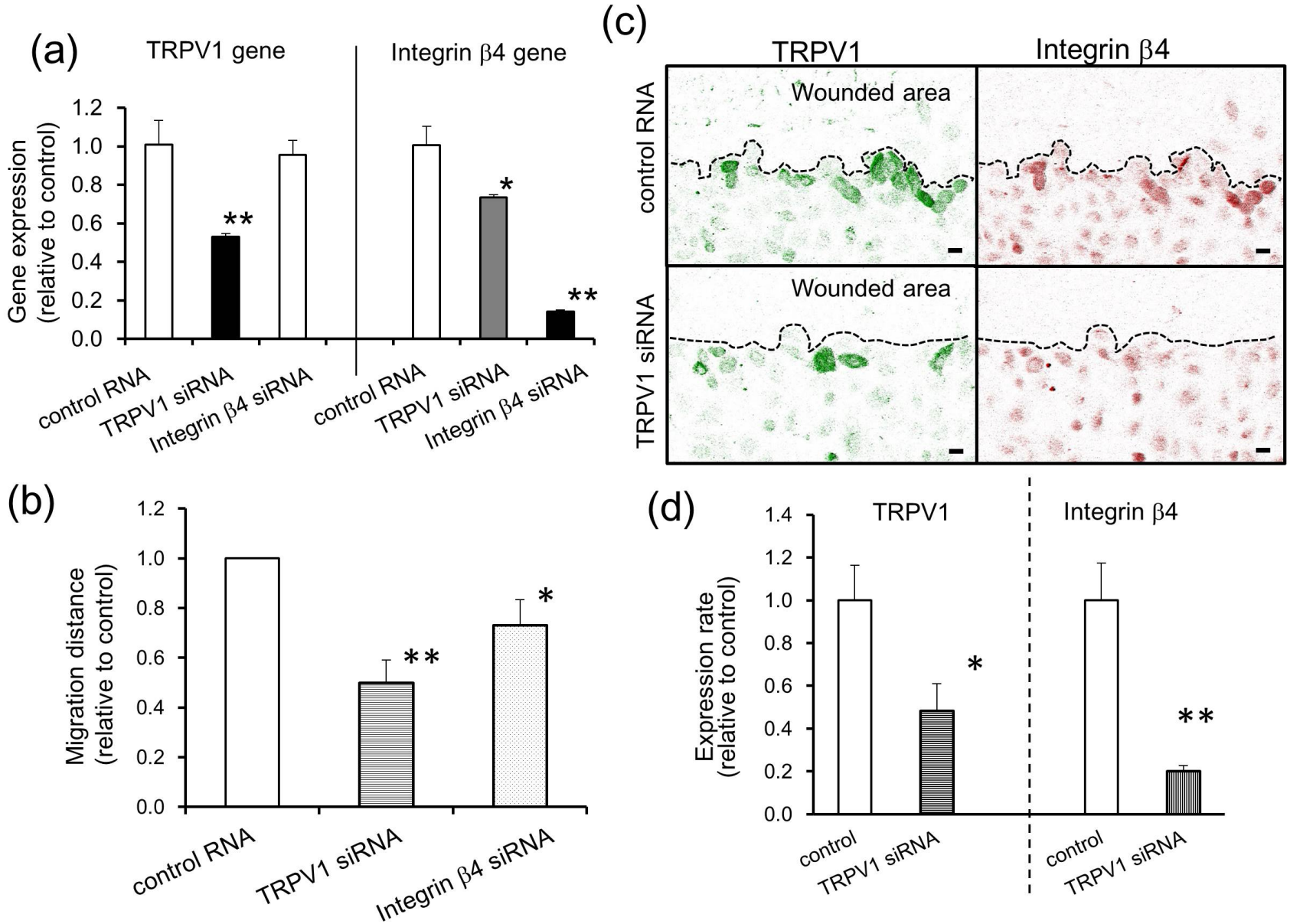
(a)
TRPV1 gene
Integrin β4 gene
Gene expression (relative to control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
*
**
control RNA
TRPV1 siRNA
Integrin β4 siRNA
control RNA
TRPV1 siRNA
Integrin β4 siRNA
(c)
TRPV1
Integrin β4
control RNA
TRPV1 siRNA
Wounded area
Wounded area
(b)
Migration distance (relative to control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
*
control RNA
TRPV1 siRNA
Integrin β4 siRNA
(d)
TRPV1
Integrin β4
Expression rate (relative to control)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
control
TRPV1 siRNA
control
TRPV1 siRNA

Fig. 4

SUPPLEMENTARY INFORMATION

Figure S1. Representative images photographed at 0 h and 12 h after the scratch wounding. The migration distance of the leading edge of the monolayer was estimated based on the wound area. Scale bar: 100 μm.

Figure S2. Decrease in expression of TRPV1 (green) and integrin β4 (red) induced by AG1478 presented as 3D images of immunofluorescence. Scale bar: 20 μm.

Figure S3. AMG9810 decreased the expression of integrin β4 (red) without the down-regulation of TRPV1 (green) at the wound edge. Scale bar: 50 μm.

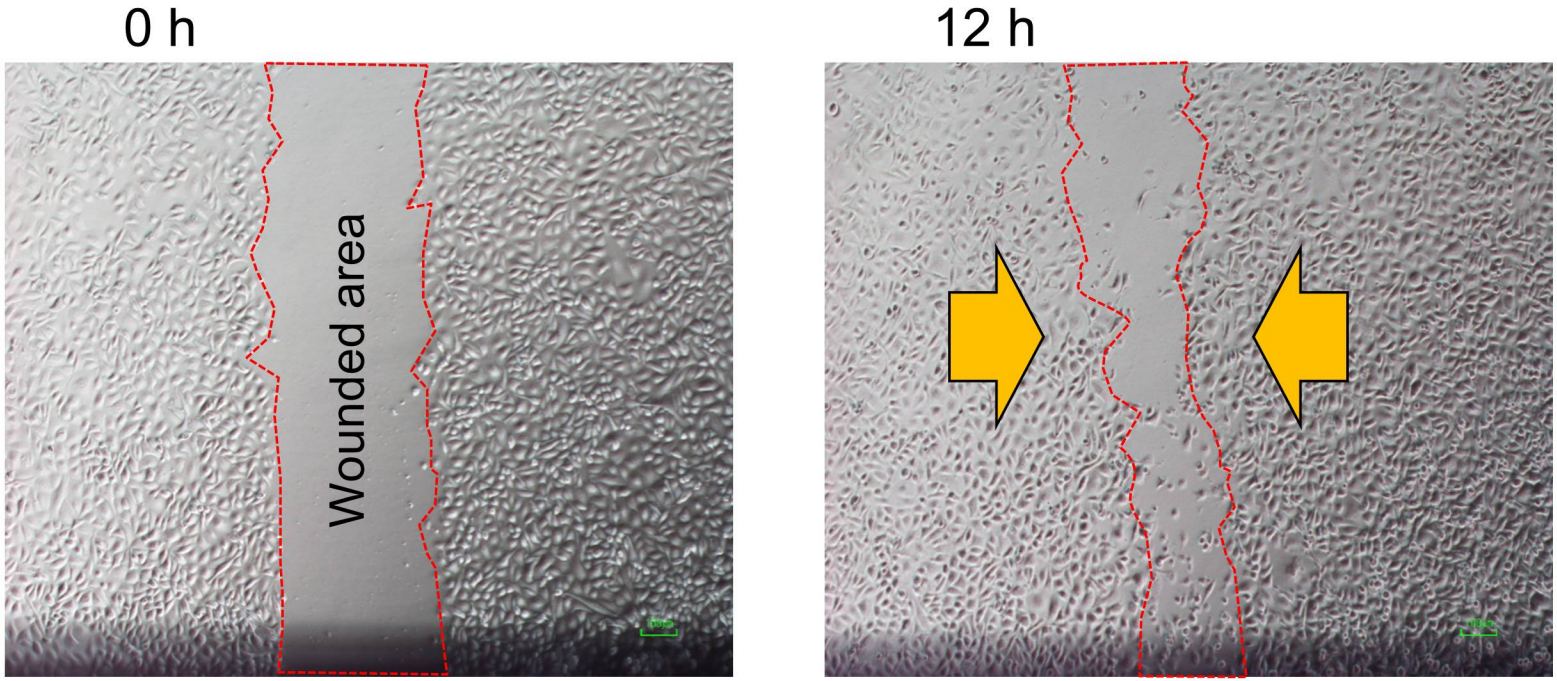

Fig. S1

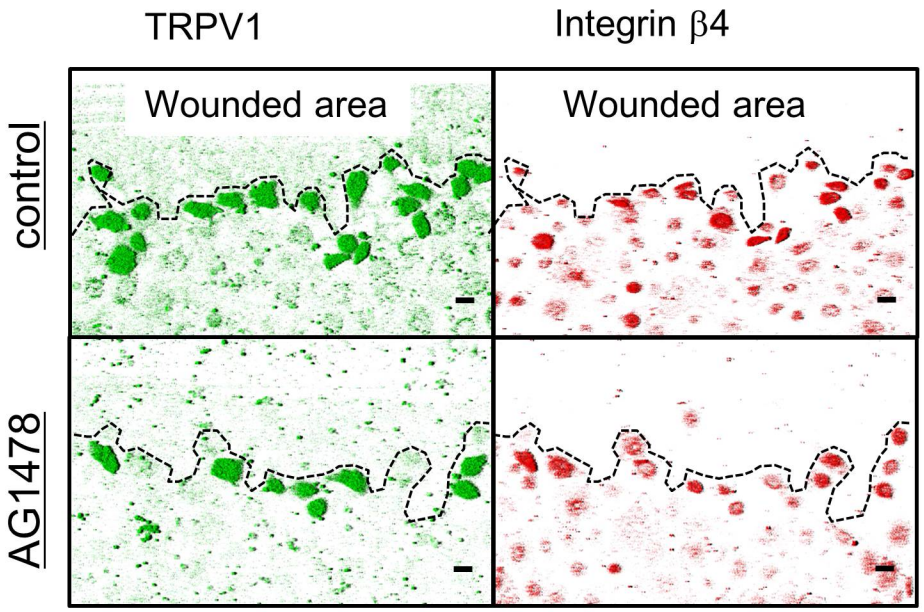
TRPV1
Integrin β4
Wounded area
Wounded area
control
AG1478

Fig. S2

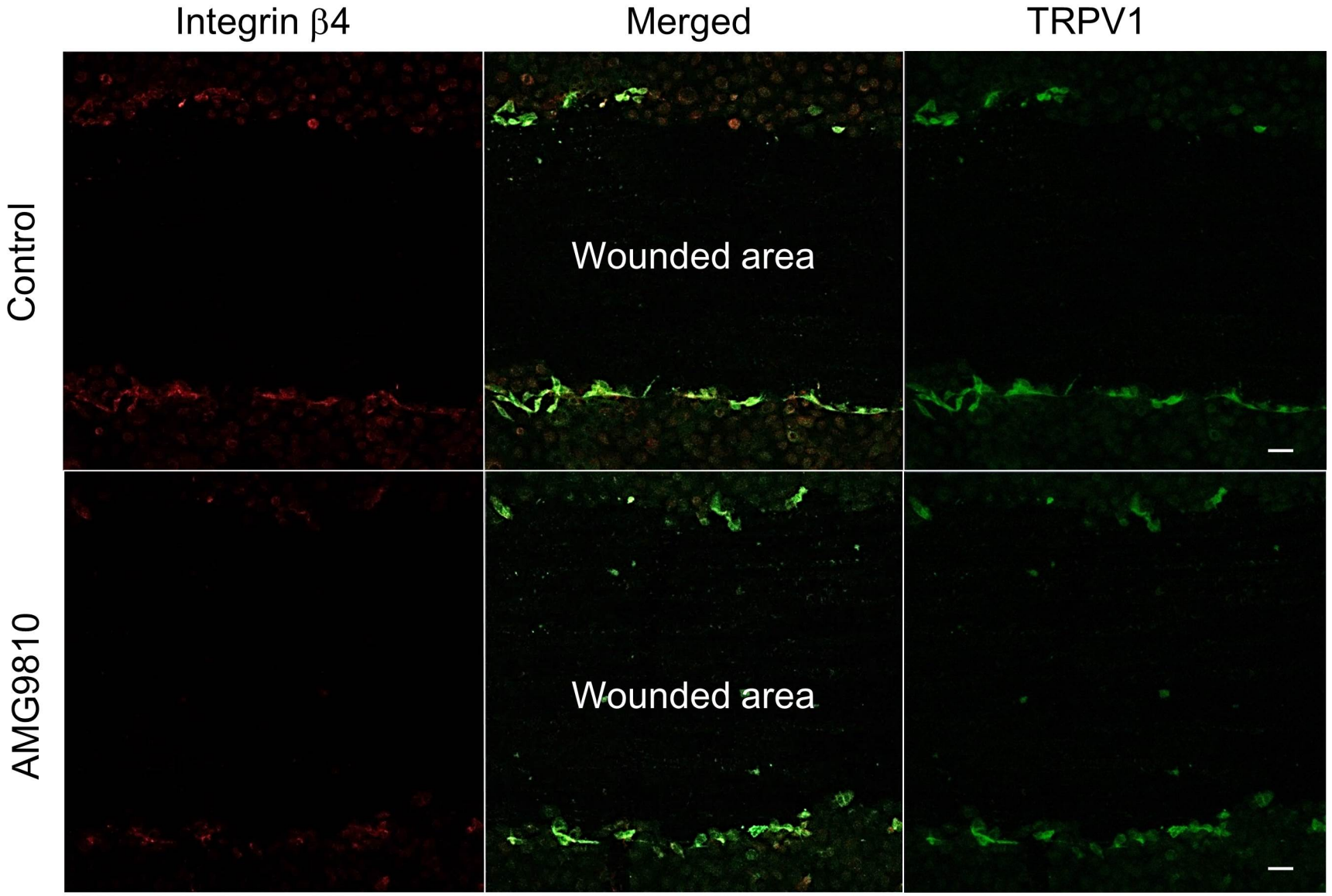
Integrin β4
Merged
TRPV1
Control
AMG9810
Wounded area
Wounded area

Fig. S3