Epithelial-mesenchymal transition in oral cancer cells induced by prolonged and persistent *Fusobacterium nucleatum* stimulation

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Abbreviations:

EMT, Epithelial-mesenchymal transition

F. nucleatum, *Fusobacterium nucleatum*

DEX, Dexamethasone

SCC, squamous cell carcinoma

Abstract

Objectives: Several studies have reported the effects of *Fusobacterium nucleatum* stimulation on oral cancer cells. However, given that these studies typically span a stimulation period of three days to eight days, the *in vitro* studies conducted to date may not fully mimic the oral cancer environment, which involves constant exposure to oral commensal bacteria. This study aimed to elucidate the effects of prolonged and persistent *Fusobacterium nucleatum* infection on oral cancer cells.

Methods: Human tongue squamous cell carcinoma (SCC) cells were continuously stimulated with *Fusobacterium nucleatum* for two or four weeks, then experimentally evaluated.

Results: Prolonged, persistent *Fusobacterium nucleatum* stimulation increased the cells' proliferative, invasive, and migratory capacities, decreased their expression of epithelial markers, and increased their expression of mesenchymal markers progressively with time. The cells also adopted a spindle-shaped morphology and cell-to-cell contact dependence was progressively lost, suggesting time-dependent occurrence of epithelial-mesenchymal transition. Furthermore, mRNA levels of CD44, a cancer stem cell marker, were time-dependently upregulated. When SCC cells were stimulated with *Fusobacterium*

nucleatum for four weeks in the presence of dexamethasone, *Fusobacterium nucleatum* induced epithelial-mesenchymal transition was inhibited.

Conclusions: Epithelial-mesenchymal transition in human tongue SCC cells was time-dependently induced by prolonged, persistent *Fusobacterium nucleatum* stimulation and inhibited by dexamethasone. Routine decontamination of the oral cavity may be crucial for controlling tumor invasion and metastasis.

Keywords: Oral cancer, *Fusobacterium nucleatum*, Epithelial-mesenchymal transition (EMT), dexamethasone.

1. Introduction

In 2018, 354,864 new cases of oral cancer were reported, accounting for 2 % of all malignancies [1]. The prognosis of oral cavity cancer is poor, with a 5-year survival rate of approximately 50–60 % and 177,384 deaths in the same year [1]. Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose cell polarity and intercellular adhesion and transform into mesenchymal-like cells, resulting in increased migration and invasive capacity, which promote tumor growth and metastasis, thereby worsening cancer prognosis [2]. Therefore, it is desirable to inhibit the EMT to improve the survival prognosis of patients with oral cancer.

Recently, an association between *Fusobacterium nucleatum* and malignant tumors has been reported in large numbers, particularly in colon [3], stomach [4], pancreatic [5], and oral cavity cancers [6]. *F. nucleatum* is an oral commensal anaerobic Gram-negative rod responsible for various dental infections including periodontitis [7]. Several studies have reported that *F. nucleatum* is detected significantly more frequently in patients with oral squamous cell carcinoma than in healthy individuals [8]. Moreover, chronic inflammation causes cell cycle arrest, cytoplasmic distention, and chromatin fragmentation [9] and is closely associated with cancer development. Because *F. nucleatum* is one of the causative factors of

chronic inflammation typified by periodontitis, *F. nucleatum* may aggravate oral carcinogenesis and cancer progression [10,11].

Recently, several studies have reported that *F. nucleatum* stimulation induces EMT in colon cancer cells and its underlying mechanisms [12–17]. However, only a few studies have reported that stimulation with oral commensal bacteria induces EMT in oral cancer cells. Although several studies have reported the effects of oral microorganisms on EMT in oral cavity cells, the cell stimulation period ranged from three days to a maximum of eight days [18–22]. Therefore, the in vitro studies conducted to date may not be sufficient to mimic the environment of oral cancer, which involves constant exposure to oral commensal bacteria.

In this study, we investigated the effects of prolonged and persistent *F. nucleatum* stimulation on oral cancer cells and EMT.

2. Material and Methods

2.1 Cell line and cell culture

HSC-3 cells (JCRB0623, human tongue squamous cell carcinoma) were provided by the National Institute of Biomedical Innovation, Health, and Nutrition (JCRB Cell Bank, Osaka, JP). HSC-3 cells were cultured in

minimum essential medium (MEM; Sigma-Aldrich, St. Louis, MO, US) containing 10 % heat-inactivated fetal bovine serum FBS (Cell Culture Technologies, Gravesano, Switzerland), 100 U/mL penicillin, and 100 mg/mL streptomycin (MEM complete) at 37 °C with 5 % CO_2.

2.2 Bacteria strain and growth condition

F. nucleatum strain ATCC10953 (American Type Culture Collection, Manassas, VA, US) was cultured anaerobically (85 % N_2, 10 % H_2, 5 %CO_2) at 37 °C in Brain Heart Infusion broth (Becton Dickinson, Franklin Lakes, US), supplemented with hemin (5 μg/mL), and menadione (1 μg/mL), for 24 h, and stored in a -70 °C freezer until just before use.

2.3 Establishment of the stimulation model

To mimic chronic inflammation caused by *F. nucleatum* in the oral cavity, HSC-3 cells were stimulated with *F. nucleatum* at a count of 1,000 bacteria per cell twice a week for four weeks at 37 °C with 5 % CO_2 in a 10 cm dish. Specifically, cells were seeded at 1.0×10^6 cells in a 10 cm dish and incubated to allow the cells to adhere to the plate. After 24 h, to achieve a count of 1,000 bacteria per cell, the wells were washed three times with sterile phosphate-buffered saline (PBS), and then PBS was replaced with 10 ml of MEM

containing 1.0×10^8 /ml of *F. nucleatum* (stimulation media). The stimulation media was adjusted by adding 1.0×10^{11} /ml of *F. nucleatum* frozen stock to MEM complete without antibiotics. This was repeated every three or four days. The cells were then stimulated for two weeks or four weeks. During this period, the cells that were unable to attach to the surface of the dish after trypsin treatment were removed by washing with PBS. Consequently, cells that stopped proliferating under *F. nucleatum* exposure, which should have been dead cells, were removed and live cells were continually exposed to *F. nucleatum* stimulation. To examine cell morphological changes, the cells stimulated for 4-, 2-week, or no stimulation were trypsinized, harvested, and inoculated in a 10 cm dish (1.0×10^6 /dish). The cells were allowed to attach to the dishes for 24 h. The medium was then replaced with stimulation medium at a count of 1,000 bacteria per cell, and the cells were observed using a phase-contrast microscope. In some experiments, cells were stimulated with or without *F. nucleatum* for four weeks in the presence of dexamethasone (DEX, an NF-κB inhibitor).

2.4 Cell counting Kit-8 assay

Cell proliferation was determined using the CCK-8 assay (CCK-8; Dojindo,

Kumamoto, Japan). In brief, a highly water-soluble tetrazolium salt, WST-8, in the CCK-8 solution is reduced by dehydrogenase activity in cells to produce a yellow-colored formazan dye, which is soluble in tissue culture media. Because the amount of formazan dye produced is directly proportional to the number of living cells, cell proliferation can be measured by measuring the absorbance of the medium. Cells stimulated for 4-, 2-week, or not stimulated, were washed with PBS, treated with trypsin, seeded in a 96-well plate at a rate of 5,000 cells per well, and incubated for 24 h. A 10 % CCK-8 solution was added to each well and incubated for 2 h. The absorbance of the colored solution was measured at 450 nm using a microplate reader (iMark Microplate Reader; Bio-Rad). Three replicate wells for each group were set up in triplicate, and the culture medium without cells was used as the negative control.

2.5 Wound-healing assay

Cells were seeded in 6-well plates at a rate of 5.0×10^5 cells/well and grown until confluence. A linear wound was created by scratching at the center of each well using a 1,000-μL pipette tip, and the wound closure at the same site was observed after 6, 12, 18, and 24 h under a phase-contrast microscope (BZ-X800; KEYENCE, Tokyo, JP) at 10× magnification. Images were analyzed

using IMAGE J software (National Institutes of Health, Bethesda, MD, US). Three replicate wells were set up for each group and the experiment was conducted in triplicate. Wound closure (%) was calculated by the following equation: Wound closure (%) = [Total vacant area (0 h) − Total vacant area (X h)]/Total vacant area (0 h) × 100.

2.6 Transwell migration assay

Cells stimulated for 4-, 2-weeks, or not stimulated were washed with PBS, treated with trypsin, and then the cells were suspended in 300 μL of a serum-free MEM on the upper chamber (1.0×10^4 cells/ chamber, Transparent PET, Membrane 24 Well 8.0 μm pore size; FALCON, Durham, USA), and 600 μL of MEM complete was added in the lower chamber. After 24 h of incubation, cells on the upper surface of the membrane in the upper chamber were removed by scraping away with a wet cotton swab, whereas cells that migrated through the pores to the lower surface of the membrane were stained with 2 % crystal violet and the migrated cells observed under a microscope (TF50-B; Kenis, Osaka, JP). The images obtained were analyzed using IMAGE J software. Three replicate wells were set up for each group and the experiment was conducted in triplicate.

2.7 Western Blotting

Cell lysates were collected and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were then transferred to polyvinylidene fluoride membranes and molecules related to EMT were detected. The primary antibodies used were as follows: anti-β-actin (clone AC15) (1:5000) (Sigma-Aldrich), Purified anti-human CD324 (E-cadherin) (1:1000) (Bio-Legend, San Diego, US), Purified anti-CD325 (N-cadherin) (1:1000) (Bio-Legend), Purified anti-cytokeratin (pan reactive) (1:1000) (Bio-Legend), snail (C15 D3) rabbit mAb (1:1000) (Cell Signaling Technologies). Protein bands were detected using appropriate secondary antibodies and Luminata Forte Western HRP Substrate (Millipore Sigma, Darmstadt, Germany) and analyzed using LAS-1000 (Fujifilm, Tokyo, JP).

2.8 Real-time RT-PCR

Total RNA was extracted using the ReliaPrep RNA Miniprep System (Promega, Madison, WI, US), and cDNA was synthesized from 1 μg of total RNA using the Revertra Ace qPCR Master Mix with gDNA Remover (TOYOBO) in reactions with the following conditions: 37 °C 15 min and 50 °C 5 min for reverse transcription, 98 °C 5 min for enzyme inactivation.

Real-time PCR was performed using a CFX Opus 96 (Bio-Rad) with cDNA prepared using KOD SYBR qPCR Mix (TOYOBO). PCR reactions were performed as follows: 98 °C for 2 min, followed by 40 cycles of 98 °C for 10 s, 65 °C for 10 s, and 68 °C for 30 s. The primer sequences used are as follows: β-actin: 5'-GGCCAACCGCGAGAAGAT-3' (forward), and 5'-CGTCACCGGAGTCCATCA-3' (reverse), CD44; 5'-TGAATATAACCTGCCGCTTTG-3' (forward), and 5'-GCTTTCTCCATCTGGGCCAT-3' (reverse), CD133; 5'-GAGTCGGAAACTGGCAGATAGCA-3' (forward), and 5'-ACGCCTTGTCCTTGGTAGTGTTG-3' (reverse). Three replicate wells were set up for each group and the experiment was conducted in triplicate. Each value was evaluated using the ΔΔCT method.

2.9 Statistical analysis

All data were obtained from at least three separate experiments. Statistical differences between the groups were determined using Tukey's range test. Statistical significance was set at $p<0.05$.

3. Results

3.1 Prolonged and persistent *F. nucleatum* stimulation increases the proliferative capacity of HSC-3 cells in a time-dependent manner

The CCK-8 assay revealed a time-dependent increase in HSC-3 cell proliferation following *F. nucleatum* stimulation. The proliferation of the cells stimulated for 4- and 2-week stimulations was significantly increased compared to that of the unstimulated cells. Moreover, cell proliferation after 4-week stimulation was significantly higher than that after 2-week stimulation (Fig.1).

3.2 Prolonged and persistent *F. nucleatum* stimulation increases the invasive and migratory capacities of HSC-3 cells in a time-dependent manner

A wound-healing assay showed that wound closure was promoted in a time-dependent manner after *F. nucleatum* stimulation. Six hours after scratching, although no significant differences were observed, wound closure tended to increase in a time-dependent manner. Twelve hours after scratching, wound closure levels at 4- and 2-week stimulation were significantly increased compared to those with no stimulation. In addition, the wound closure level at 4-week stimulation was significantly higher than that after 2-week stimulation. Eighteen hours after scratching, wound closure levels at 4-week and 2-week

stimulation were significantly higher than those with no stimulation (Figs.2a and 2b). The results of the Transwell migration assay showed that the average migration area increased in a time-dependent manner after *F. nucleatum* stimulation. The average migration area in the 4-week stimulation group was significantly larger than that in the no-stimulation group (Fig.2c). Additionally, the average migration area in the 4-week stimulation group was significantly larger than that in the 2-week stimulation group (Fig.2d).

3.3 Prolonged and persistent *F. nucleatum* stimulation induces a time-dependent decrease in epithelial markers and a time-dependent increase in mesenchymal markers in HSC-3 cells

Western blotting revealed that the expression levels of the epithelial markers E-cadherin and Cytokeratin were downregulated in a time-dependent manner, whereas those of the mesenchymal markers N-cadherin and Snail were upregulated (Fig.3).

3.4 Prolonged and persistent *F. nucleatum* stimulation induces time-dependent EMT in HSC-3 cells

Phase-contrast microscopy images of HSC-3 cells after 4-week, 2-week, and without stimulation revealed that *F. nucleatum* stimulation induced a time-

dependent transformation into a slender and elongated morphology, along with cell-to-cell separation. In particular, the morphological differences among HSC-3 cells after 4-week, 2-week, and no stimulation are clearly shown in the magnified images. The non-stimulated cells were mostly round to oval, whereas many of the stimulated cells were spindle-shaped (Fig.4). This suggests loss of cell polarity and intercellular adhesion. Additionally, rod-shaped *F. nucleatum* particles were observed around the cells, suggesting that these cells were affected by *F. nucleatum*. Taken together, prolonged and persistent *F. nucleatum* stimulation induced a time-dependent EMT in HSC-3 cells, as indicated by increased proliferative, invasive, and migratory capacities, decreased expression of epithelial markers, and increased expression of mesenchymal markers in HSC-3.

3.5 Prolonged and persistent *F. nucleatum* stimulation induces HSC-3 cells to acquire cancer stem cell-like properties in a time-dependent manner

Since HSC-3 cells acquire the characteristics of mesenchymal cells and may have acquired the properties of cancer stem cells through prolonged and persistent *F. nucleatum* stimulation, the expression levels of CD44 and CD133, which are markers of cancer stem cells, were analyzed using real-time RT-

PCR. The mRNA level of CD44 was upregulated in a time-dependent manner, and the mRNA level at 4-week stimulation was significantly higher than that at no stimulation. Although no significant differences were observed, the mRNA level of CD133 were upregulated in a time-dependent manner (Fig.5).

3.6 DEX impairs EMT of HSC-3 cells induced by prolonged and persistent *F. nucleatum* stimulation

To examine the effects of DEX on *F. nucleatum*-stimulated HSC-3 cells, the cells were incubated for four weeks with or without *F. nucleatum* in the presence or absence of DEX. In the presence of DEX, HSC-3 cells did not show any increase in proliferative, invasive, or migratory capacity, regardless of *F. nucleatum* stimulation, and there were significant decreases compared to those stimulated with *F. nucleatum* alone (Figs.6a, b, c, and d). Furthermore, HSC-3 cells stimulated with DEX alone and DEX plus *F. nucleatum* did not induce significant changes in the expression levels of E-cadherin, Cytokeratin, N-cadherin, or Snail, whereas HSC-3 cells stimulated with *F. nucleatum* alone induced downregulation of the expression levels of E-cadherin, Cytokeratin and upregulated the expression levels of N-cadherin and Snail, suggesting that DEX exerted inhibitory effects on the EMT induced by *F. nucleatum*

stimulation (Fig.6e). In addition, no transformation into a slender or elongated morphology or cell-cell separation was observed (Fig.6f).

4. Discussion

The effects of prolonged and persistent stimulation of tumors by *F. nucleatum* were confirmed in this study. As *F. nucleatum* is an anaerobic bacterium, it cannot survive under cell culture conditions. Therefore, it can be said that the present study revealed not the effects of *F. nucleatum* infection but the effects of *F. nucleatum*-derived components on oral cancer cells. To our knowledge, this is the first study to examine the effects of prolonged and persistent *F. nucleatum* stimulation on oral tumor cells. In this study, EMT was induced in a time-dependent manner even after 4-week *F. nucleatum* stimulation, suggesting that oral cancer cells continue to be adversely affected by prolonged and persistent *F. nucleatum* stimulation. To our knowledge, this is the first study to show that prolonged and persistent *F. nucleatum* stimulation causes EMT in oral cancer cells in a time-dependent manner. Because periodontitis is a chronic disease, it is difficult to reconstruct its condition *in vitro*, and *F. nucleatum* stimulation for four weeks may not reflect chronic inflammation. However, since we revealed that

there were continuous malignant changes in the tumor cells even after *F. nucleatum* stimulation for four weeks, this may suggest that the longer the stimulation period with *F. nucleatum*, the greater the induction of EMT cells may be induced. This is an important finding regarding the aggravation of oral cancer caused by *F. nucleatum* stimulation. Therefore, routine decontamination of the oral cavity to prevent prolonged and persistent *F. nucleatum* stimulation may be important for controlling tumor invasion and metastasis.

CD44 is known as a cancer stem cell marker for squamous cell carcinoma of the head and neck [23], and its mRNA level is significantly and time-dependently elevated by *F. nucleatum* stimulation in this study. These findings indicate that cells subjected to prolonged and persistent *F. nucleatum* stimulation have poor prognostic properties, including increased proliferative capacity, invasive potential, and acquisition of mesenchymal cell characteristics. CD133 is reported to be a cancer stem cell marker found in several cancers [24], although the failure to detect its positivity in oral cancer has also been reported[25]. In this study, the mRNA level of CD133 tended to increase in a time-dependent manner, suggesting the possibility that the cells acquired cancer stem cell characteristics.

Studies on colon cancer cells have reported that *F. nucleatum* promotes EMT by

inducing Annexin A1 and activating the Wnt/β-catenin signaling pathway through *Fusobacterium* adhesin A (FadA) and E-cadherin [12,13]. FadA is one of the most characteristic virulence factors of *F. nucleatum* and can bind to host cells via E-cadherin [26]. Therefore, FadA may also be a causative agent of EMT in oral cancer. Additionally, it has been observed that *F. nucleatum* activates the Toll-like receptor (TLR)4/ MyD88/NF-κB pathway, leading to the promotion of EMT [14–16] and chemotherapy resistance [17].

In oral cancer cells, studies have reported that *F. nucleatum* suppresses apoptosis by activating the Wnt5a/Nuclear factor of activated T-cells (NFAT) pathway and promotes the migration of oral cancer cells, resulting in cisplatin resistance [27]. Thus, the underlying mechanism may be similar to that observed in colon cancer cells. *F. nucleatum* and *Porphyromonas gingivalis* stimulation activates the JAK-STAT pathway via TLRs to regulate the expression of genes that promote cell proliferation, angiogenesis, metastasis, and invasion, thereby promoting cancer progression [28].

In oral cancer, there are no reports on the effects of stimulation with *F. nucleatum* alone on EMT induction via the JAK-STAT pathway and its downstream phosphatidylinositol-3 kinase (PI3K) pathway. Therefore, we used DEX to

investigate the association between *F. nucleatum* and JAK-STAT, and their downstream pathway, the PI3K pathway, in oral cancer. NF-κB is known to be upregulated by activation of the PI3K pathway. According to Janeway's IMMUNOBIOLOGY, DEX binds to an intracellular steroid receptor and its complex directly interacts with NF-κB, thereby inhibiting transcription of its downstream genes [29]. A few studies have reported an association between DEX administration and malignancies. A previous study reported that DEX inhibits hypoxia-induced EMT in colon cancer cells [30]. Furthermore, DEX partially improves EMT in oral cancer when administered to cells undergoing EMT [31]. Similarly, DEX treatment inhibited EMT in HSC-3 cells induced by prolonged and persistent *F. nucleatum* stimulation. This may suggest that the PI3K pathway is associated with EMT induced by *F. nucleatum* stimulation in oral cancer, as well as its upstream JAK-STAT pathway. Therefore, the inhibition of the JAK-STAT and PI3K pathways may inhibit malignant tumor progression. Treatment with recombinant IL-6 or stable IL-6 overexpression in human tongue cancer cells induces the expression of the mesenchymal marker vimentin and promotes EMT, while suppressing E-cadherin expression via the JAK/STAT3/Snail signaling pathway [32]. However, the detailed mechanism of EMT via the JAK-STAT and

PI3K pathways has not yet been investigated and further studies on the association between *F. nucleatum* and the JAK-STAT and PI3K pathways are required.

In this study, the effects of prolonged and persistent *F. nucleatum* stimulation were examined using one cell line, HSC-3, and further studies using clinical specimens are needed in the future.

5. Conclusions

To our knowledge, this is the first study to demonstrate that prolonged and persistent *F. nucleatum* stimulation induces EMT in HSC-3 cells in a time-dependent manner. In addition, HSC-3 cells may acquire cancer stem cell characteristics in a time-dependent manner after prolonged and persistent *F. nucleatum* stimulation. Furthermore, it was suggested that the JAK-STAT pathway is involved in *F. nucleatum*-induced EMT, as EMT induction was inhibited by DEX treatment.

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Data availability statement

All data supporting the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

Not applicable.

Ethical Approval

Ethical approval was not required for this article.

Reference

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Figure Legends

Fig.1

The effects of *F. nucleatum* stimulation on the proliferation of HSC-3 cells were

determined using a CCK-8 assay. NS: not stimulated; 2 weeks: stimulated for 2 weeks; 4 weeks: stimulated for 4 weeks. ＊p<0.05

Fig.2

(a) Representative images of wound healing assay recorded at 0, 6, 12, and 18 h after scratching in HSC-3 cells at 10× magnification under a phase-contrast microscope. (b) Wound closure (%) at 6 h, 12 h, 18 h, and 24 h in HSC-3 cells. (c) Representative images of transwell migration assay recorded at 24 h after seeding HSC-3 cells at 30× magnification under a microscope. (d) Average migration area (μm^2) at 24 h for HSC-3 migration. NS; not stimulated, 2 w; stimulated for 2 weeks, 4 w; stimulated for 4 weeks. ＊p<0.05

Fig.3

The protein expression of E-cadherin, cytokeratin, N-cadherin, snail, and β-actin of HSC-3 cells. Their Fold changes were calculated after normalization to β-actin. NS; not stimulated, 2 w; stimulated for 2 weeks, 4 w; stimulated for 4 weeks.

Fig.4

Effect of *F. nucleatum* stimulation on the cell shape of HSC-3 cells. Representative images were obtained using a phase-contrast microscope. The right images are enlarged views of the square area in the left images. Arrows indicate rod-shaped *F. nucleatum*. NS;

not stimulated, 2 w; stimulated for 2 weeks, 4 w; stimulated for 4 weeks.

Fig.5

Effect of *F. nucleatum* stimulation on the mRNA expression levels of CD44 and CD133 of HSC-3 cells. Their relative quantities were determined using real-time RT-PCR. NS; not stimulated, 2 w; stimulated for 2 weeks, 4 w; stimulated for 4 weeks. ＊p<0.05

Fig.6

Effects of DEX (50 nM) on EMT induction in HSC-3 cells stimulated with *F. nucleatum.* (a) The relative cell proliferation activity in HSC-3 cells treated with DEX, with *F. nucleatum*, or with DEX and *F. nucleatum.* (b) Representative images of wound healing assay recorded at 12 h after scratching in HSC-3 cells with DEX, with *F. nucleatum*, or with DEX and *F. nucleatum* at 10× magnification under a phase-contrast microscope. NS; not stimulated. (c) Wound closure (%) at 6 h, 12 h, 18 h, and 24 h for HSC-3 cells with DEX, with *F. nucleatum*, or with DEX and *F. nucleatum*. (d) The relative average migration area of HSC-3 cells stimulated with DEX, with *F. nucleatum*, or with DEX and *F. nucleatum*. (e) The protein expressions of E-cadherin, cytokeratin, N-cadherin, snail, and β-actin of HSC-3 cells with DEX, with *F. nucleatum*, or with DEX and *F. nucleatum* were determined by Western blotting. Their Fold changes were calculated after normalization to β-actin. (f) Representative images of HSC-3 cells with DEX, with *F.*

nucleatum, or with DEX and *F. nucleatum*, and no stimulation under a phase-contrast microscope. ＊$p<0.05$

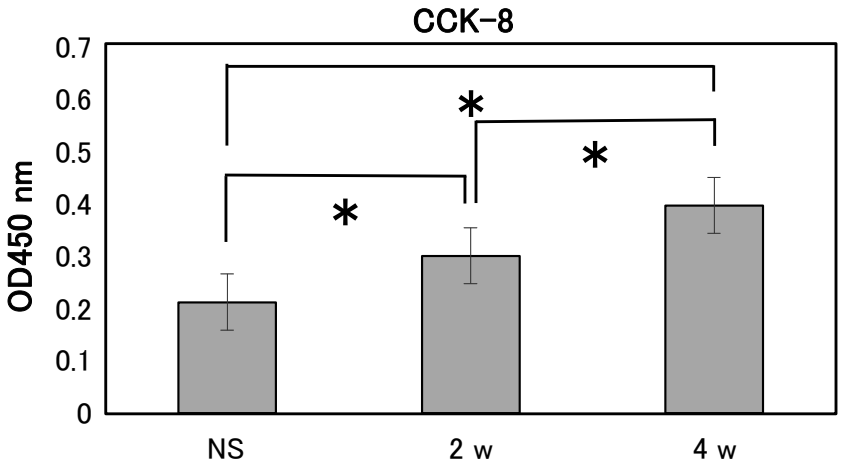


Fig. 1

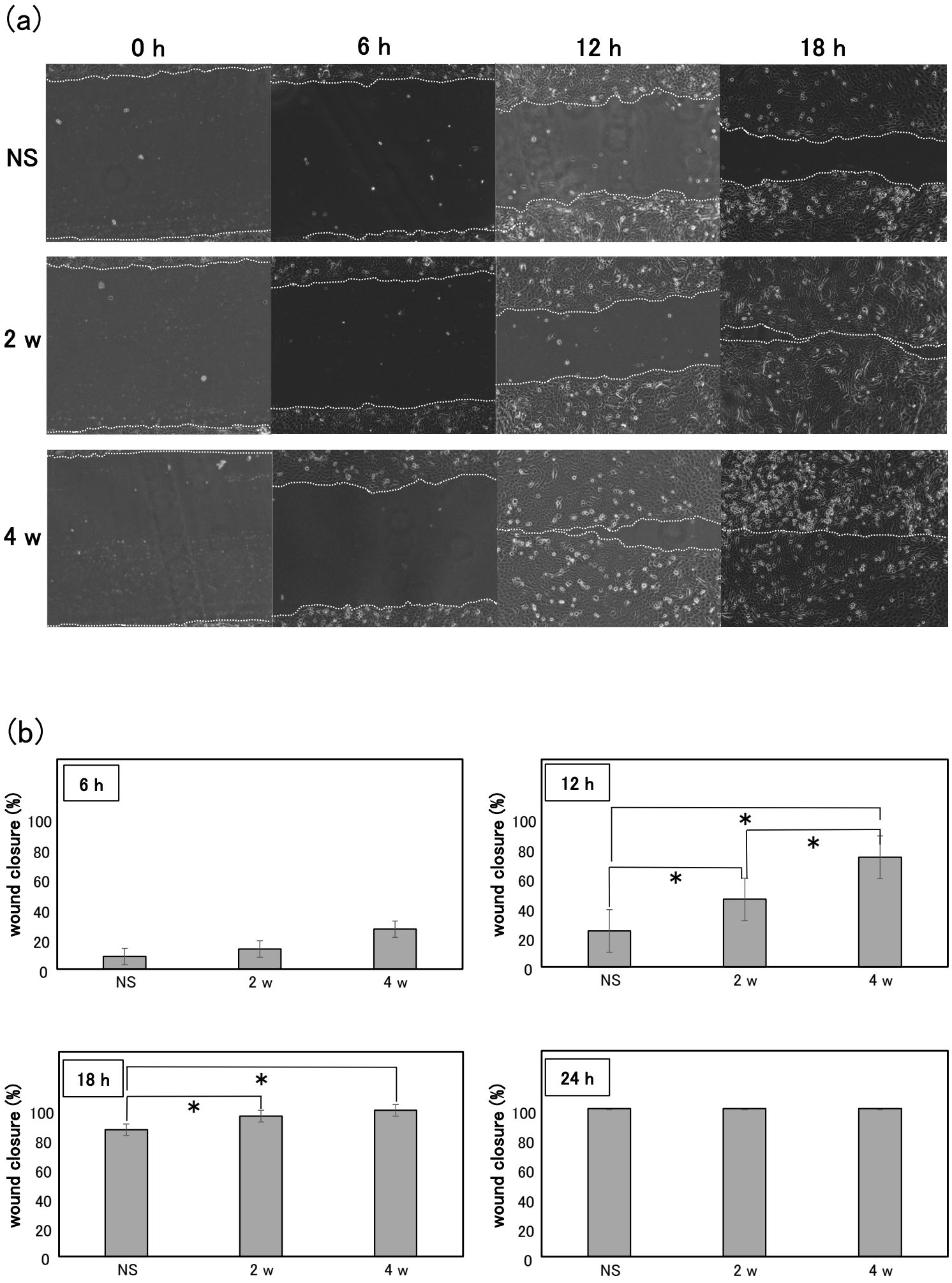
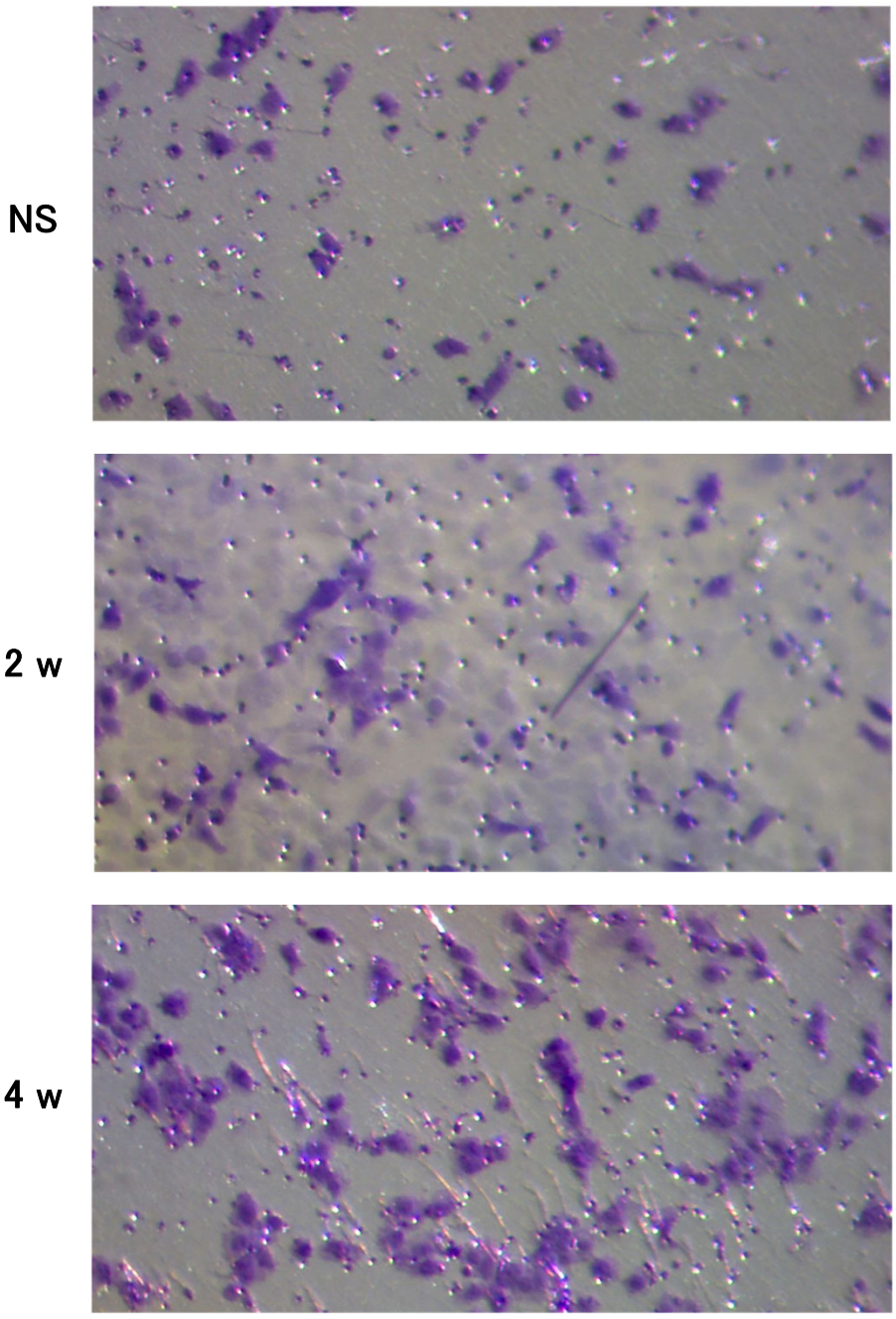

(a)
0 h
6 h
12 h
18 h
NS
2 w
4 w
(b)
6 h
12 h
18 h
24 h
wound closure (%)
0
20
40
60
80
100
NS
2 w
4 w
*

Fig. 2

(c)


NS
2 w
4 w

(d)

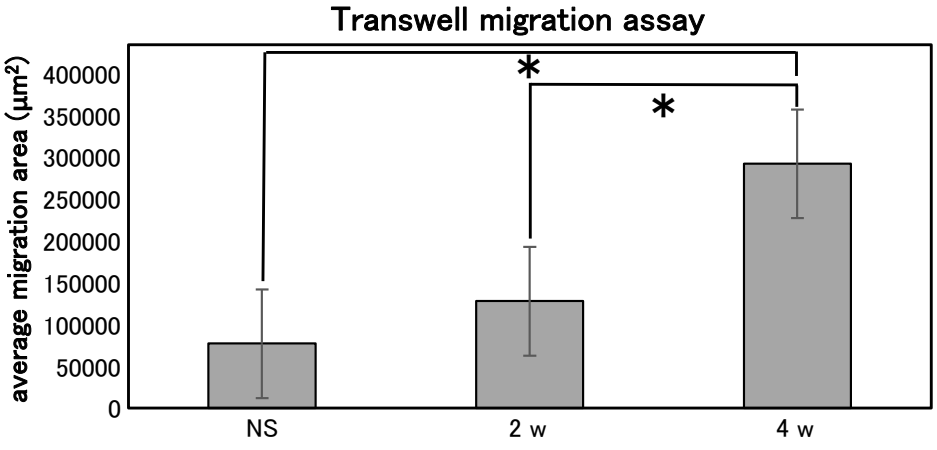

Transwell migration assay
average migration area (μm²)
400000
350000
300000
250000
200000
150000
100000
50000
0
*
*
NS
2 w
4 w

Fig. 2

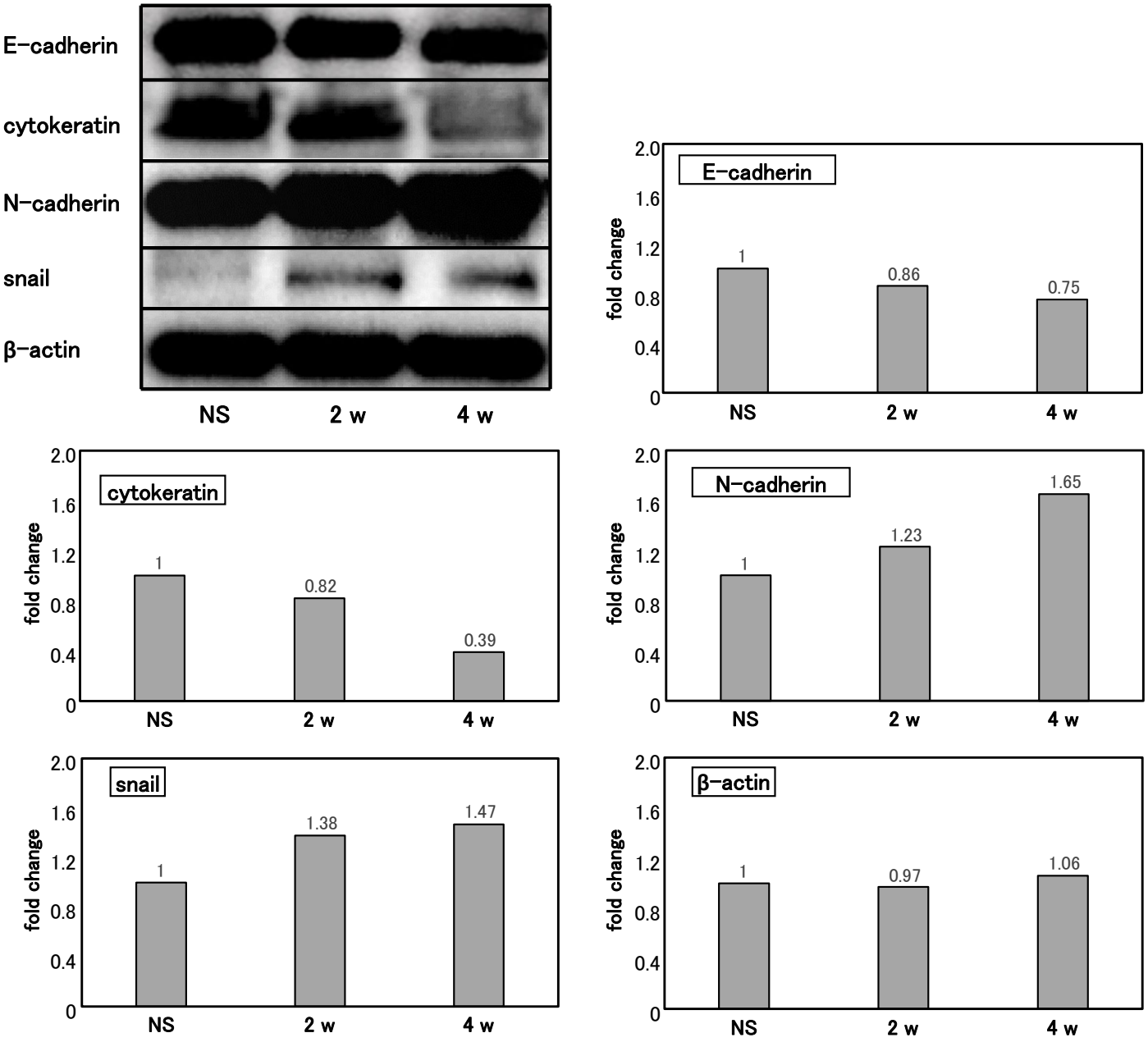

E-cadherin
cytokeratin
N-cadherin
snail
β-actin
NS
2 w
4 w
E-cadherin
fold change
2.0
1.6
1.2
0.8
0.4
0
1
0.86
0.75
NS
2 w
4 w
cytokeratin
fold change
1
0.82
0.39
NS
2 w
4 w
N-cadherin
fold change
1
1.23
1.65
NS
2 w
4 w
snail
fold change
1
1.38
1.47
NS
2 w
4 w
β-actin
fold change
1
0.97
1.06
NS
2 w
4 w

Fig. 3

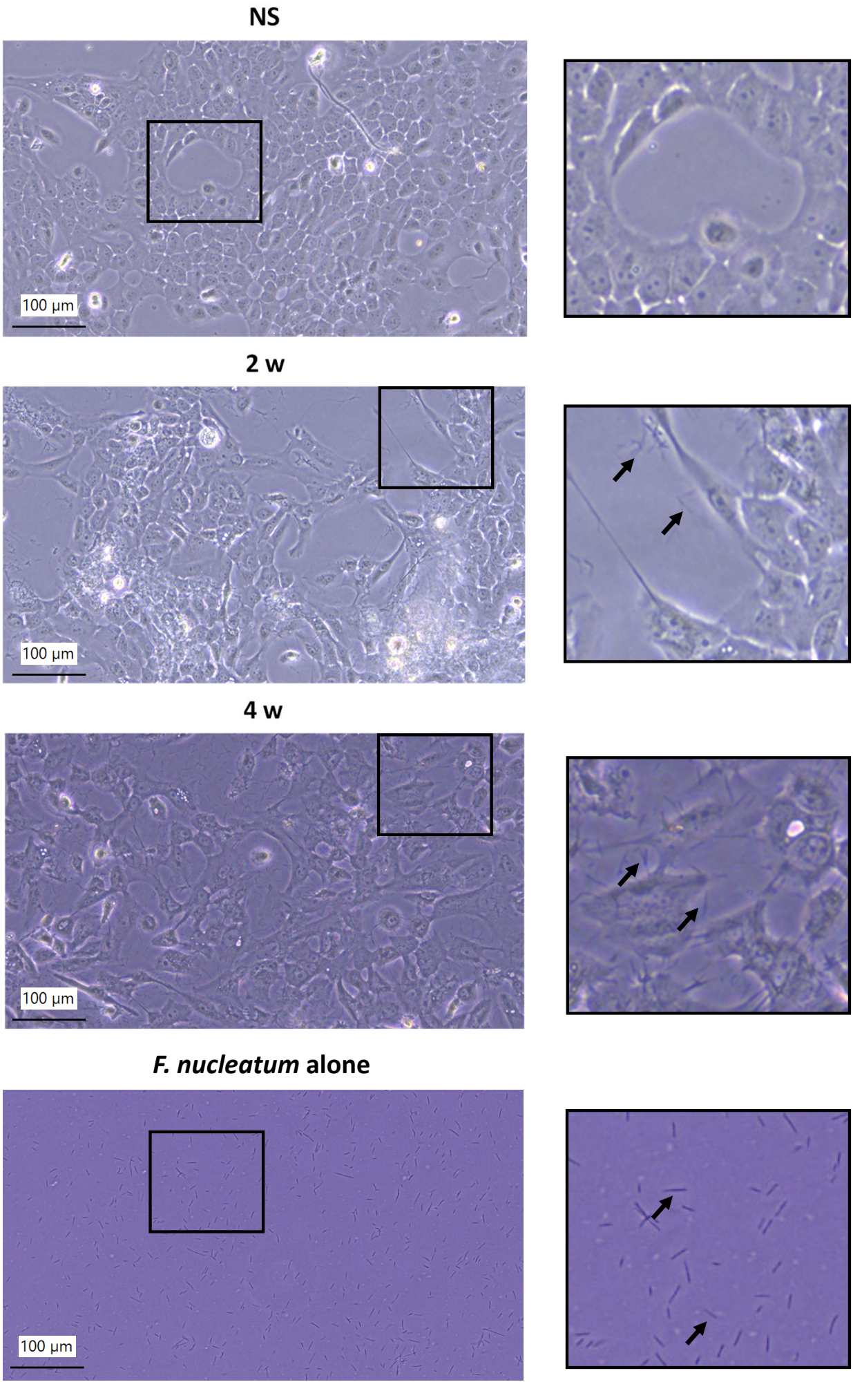


Fig. 4

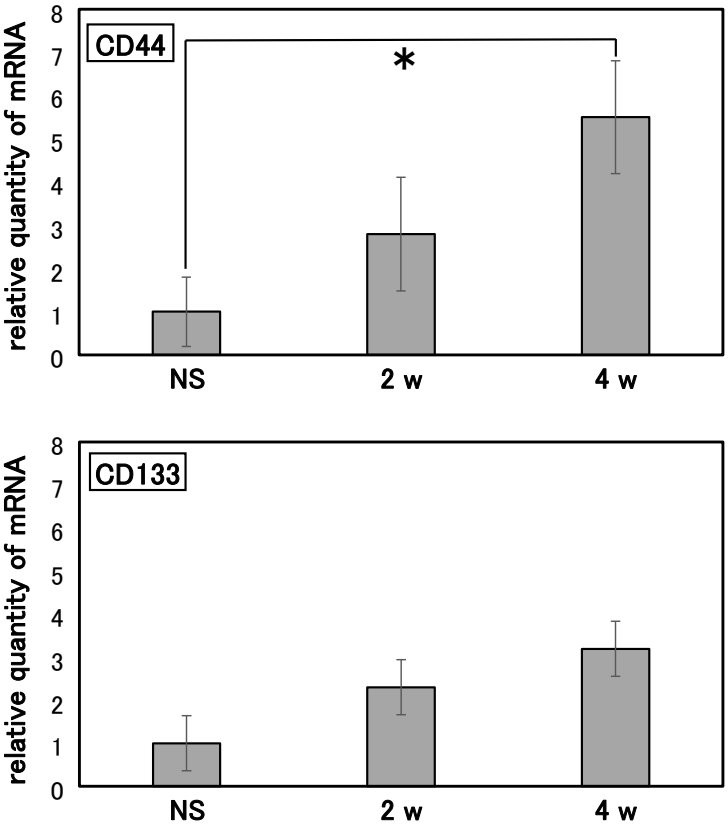

CD44
relative quantity of mRNA
0
1
2
3
4
5
6
7
8
*
NS
2 w
4 w
CD133
relative quantity of mRNA
0
1
2
3
4
5
6
7
8
NS
2 w
4 w

Fig. 5

(a)

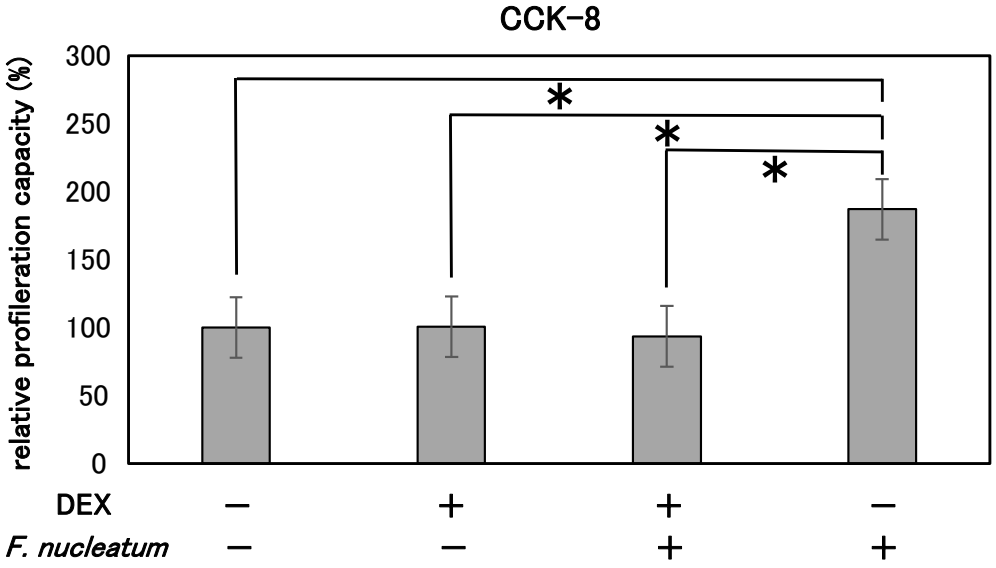

CCK-8
relative profileration capacity (%)
300
250
200
150
100
50
0
*
*
*
DEX
F. nucleatum
−
+
+
−
−
−
+
+

Fig. 6

(b)

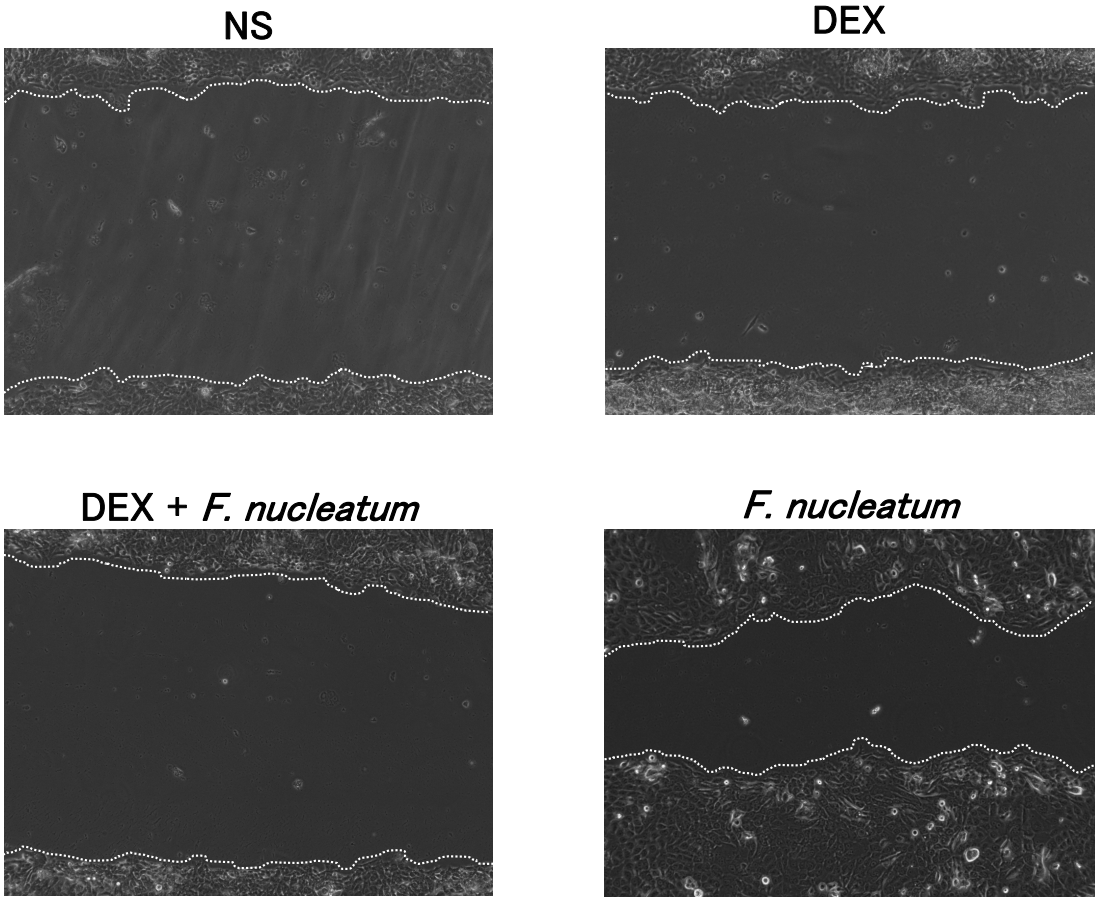
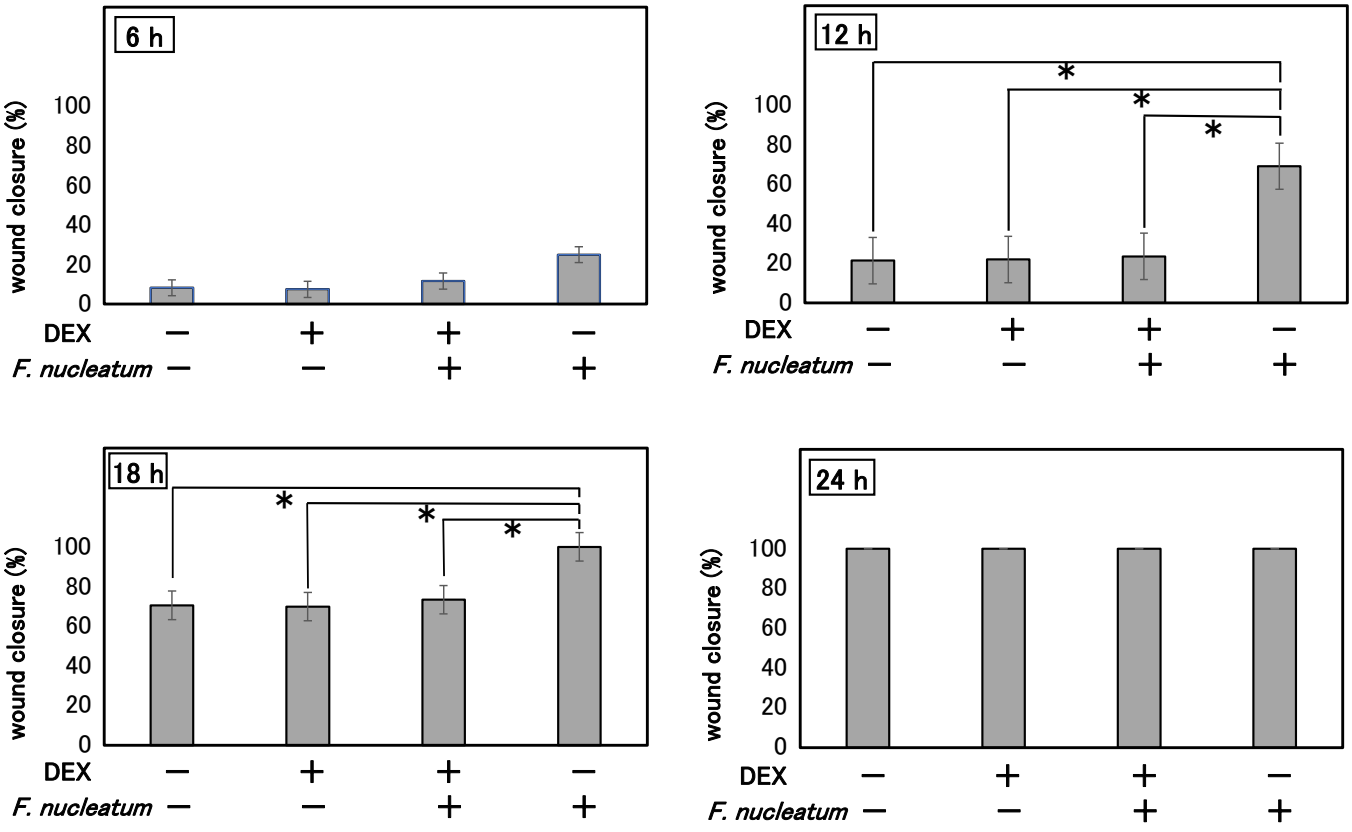


Fig. 6

(c)


6 h
12 h
18 h
24 h
wound closure (%)
0
20
40
60
80
100
DEX
F. nucleatum
−
+
*

(d)

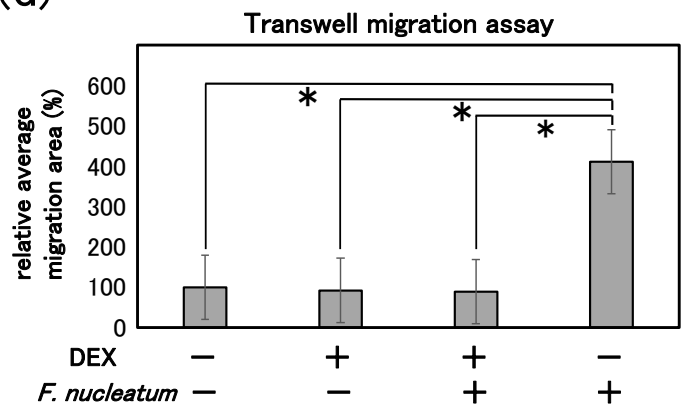

Transwell migration assay
relative average migration area (%)
0
100
200
300
400
500
600
DEX
F. nucleatum
−
+
*

Fig. 6

(e)

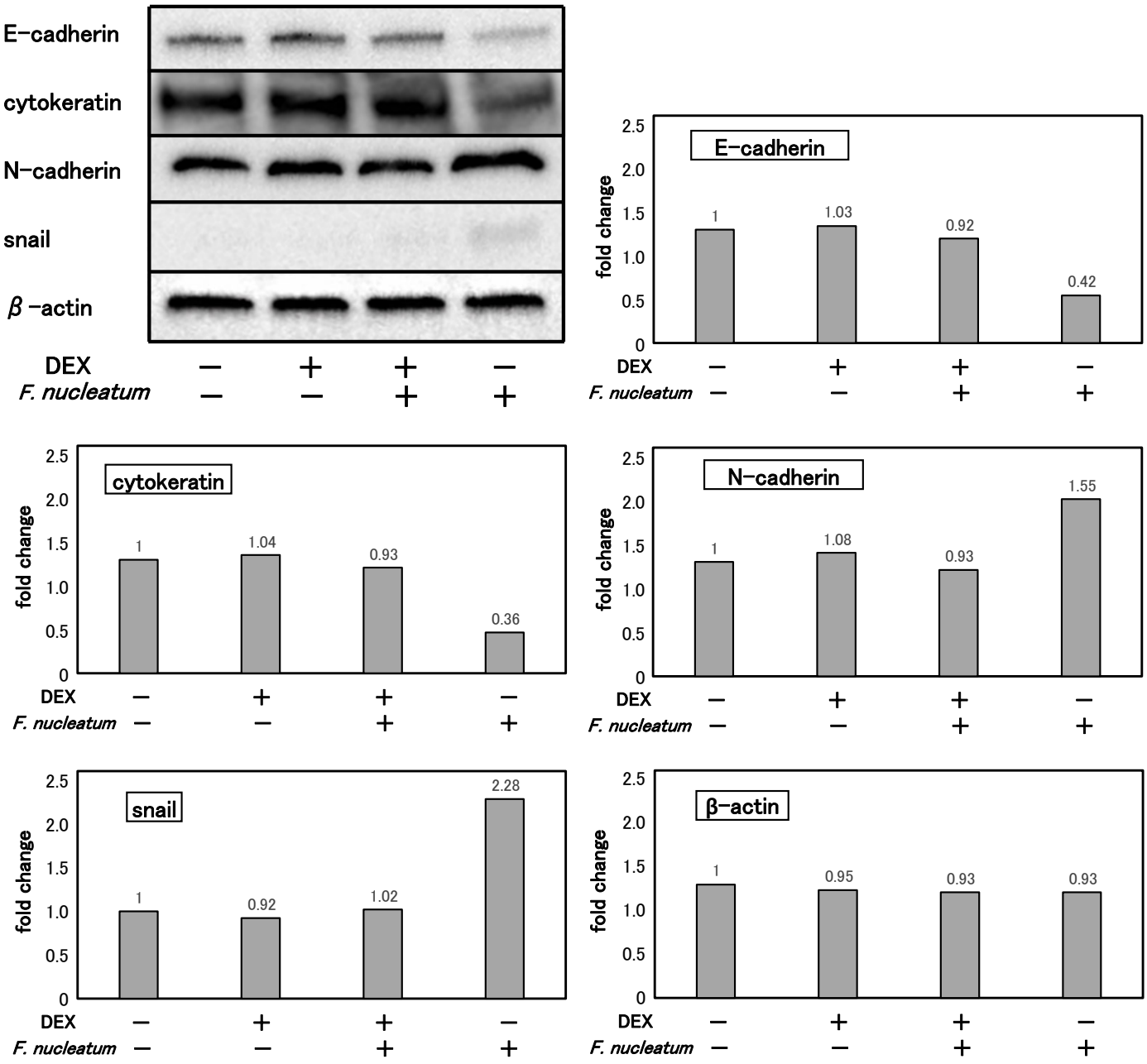

E-cadherin
cytokeratin
N-cadherin
snail
β-actin
DEX
F. nucleatum
fold change
E-cadherin
1
1.03
0.92
0.42
cytokeratin
1
1.04
0.93
0.36
N-cadherin
1
1.08
0.93
1.55
snail
1
0.92
1.02
2.28
β-actin
1
0.95
0.93
0.93

Fig. 6

(f)

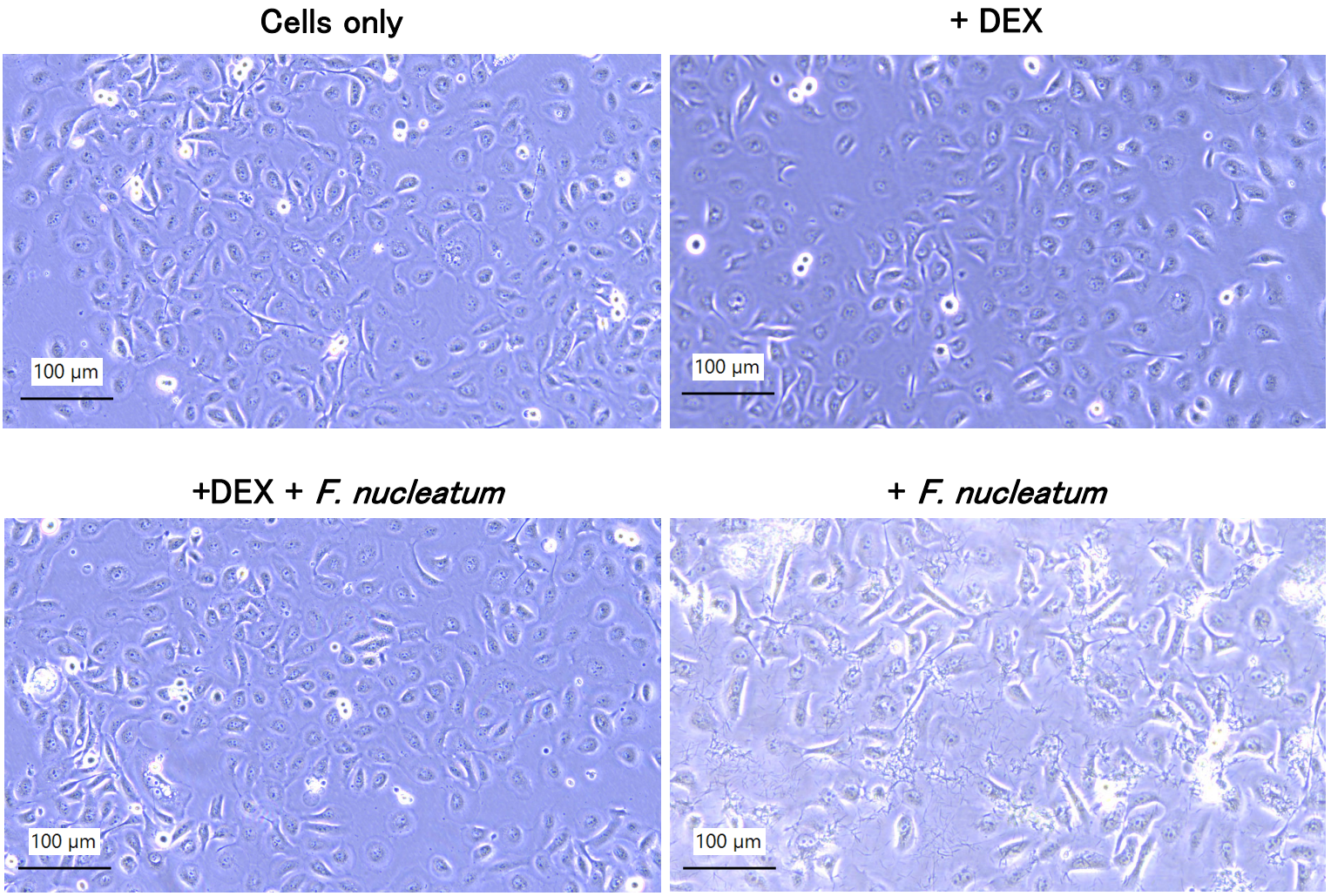

Cells only
+ DEX
+DEX + F. nucleatum
+ F. nucleatum
100 µm
100 µm
100 µm
100 µm

Fig. 6