1   **Supplementary Methods**

2   **Data information**

3   The mRNA-seq and clinical annotation data analyzed in Supplementary Fig. S2a were downloaded from

4   the TARGET data matrix (https://target-data.nci.nih.gov/Public/OS/), and among all cases, 101 had

5   mRNA-seq data and 17 were excluded because of a lack of prognostic data for survival analysis. We

6   extracted the transcripts per kilobase million (TPM) of each case using R software (version 4.1.2; The R

7   Foundation, Vienna, Austria) and performed Kaplan-Meier analysis using GraphPad Prism 6 software.

8   The cases were divided into *CD109*-high and -low groups on the basis of the cut-off value: the mean of

9   TPM.

10   We analyzed publicly available single-cell RNA-seq dataset GSE152048 [25]. We generated t-SNE

11   plots using Cell Ranger 6.1.2 and Loupe Browser 6.0.0 (10x Genomics, Inc, Pleasanton, CA, USA) and

12   annotated cell clusters based on the canonical markers used in a previous study [25].

13

14   **IL-6 stimulation of MG-63 cells**

15   In Supplementary Fig. S3c, MG-63 cells were treated with 50 ng/mL IL-6 (Bio-Techne, Minneapolis,

16   MN, USA) for the indicated times before lysis.

17

1 **Supplementary Tables**

2 **Supplementary Table S1** Antibodies used for western blot analysis

3 **(a)** Primary antibodies used for western blot analysis

Primary antibody (clone name / catalogue number)	Dilution	Vendor
Anti-CD109 (C-9)	1:500	Santa Cruz Biotechnology (Dallas, TX, USA)
Anti-β-actin (AC-74)	1:20000	Merck (Darmstadt, Germany)
Anti-SMAD2 (D43B4)	1:1000	Cell Signaling Technology (Danvers, MA, USA)
Anti-phospho-SMAD2 (138D4)	1:1000	Cell Signaling Technology
Anti-SMAD2/3 (D7G7)	1:1000	Cell Signaling Technology
Anti-phospho-SMAD3 (EP823Y)	1:2000	Abcam (Cambridge, UK)
Anti-SMAD1 (rabbit polyclonal antibody)	1:500	Cell Signaling Technology
Anti-phospho-SMAD1/5/9 (D5B10)	1:1000	Cell Signaling Technology
Anti-phospho-ERK1/2 (20G11)	1:1000	Cell Signaling Technology
Anti-ERK1/2 (rabbit polyclonal antibody)	1:1000	Cell Signaling Technology
Anti-phospho-STAT3 (D3A7)	1:2000	Cell Signaling Technology
Anti-STAT3 (79D7)	1:2000	Cell Signaling Technology

4

5 **(b)** Secondary antibodies used for western blot analysis

Secondary antibody	Vendor
Horseradish peroxidase–conjugated rabbit anti-mouse polyclonal antibody	Agilent (Santa Clara, CA, USA)
Horseradish peroxidase–conjugated swine anti-rabbit polyclonal antibody	Agilent

6

1   **Supplementary Table S2**   Antibodies used for immunohistochemistry

2   **(a)** Primary antibodies used for immunohistochemistry

Primary antibody (clone)	Retrieval	Dilution	Vendor
Anti-CD109 (C-9)	pH 9	1:100	Santa Cruz Biotechnology
Anti-phospho-SMAD1/5/8 (rabbit polyclonal)	pH 9	1:50	Merck

3

4   **(b)** Secondary antibodies used for immunohistochemistry

Secondary antibody	Vendor
EnVision+ System-HRP Labeled Polymer Anti-Rabbit	Agilent
EnVision+ System-HRP Labeled Polymer Anti-Mouse	Agilent

5

1 **Supplementary Figure legends**

2 **Supplementary Fig. S1** Histological analyses of human osteosarcomas.

3 (a) Number of cases with PS, IS, and TS for CD109. (b) Representative histological images of human

4 osteosarcoma at low magnification. H&E staining (top panels) and immunohistochemical staining with

5 anti-CD109 antibody (bottom panels) in the same area in the serial section. Each case corresponds to

6 that with the indicated intensity score in Fig. 1b. Arrowheads indicate neoplastic osteoids or bones. PS,

7 proportion score; IS, intensity score; TS, total score; H&E, hematoxylin and eosin.

8

9 **Supplementary Fig. S2** *In silico* analyses of publicly available data of osteosarcoma.

10 (a) Overall survival based on *CD109* mRNA expression was analyzed by the Kaplan–Meier method

11 using public RNA-seq data from the TARGET osteosarcoma project. (b) t-SNE plots using publicly

12 available data of 11 osteosarcoma lesions. *RUNX2* and *CD109* expressions are shown in the lower

13 panels. N.S., not significant; OS, osteosarcoma; MSC, mesenchymal stem cell.

14

15 **Supplementary Fig. S3** CD109 expression and ERK1/2 or STAT3 phosphorylation in human

16 osteosarcoma cell lines.

17 (a) Time course of ERK1/2 phosphorylation after BMP-2 stimulation in CD109 knockdown and control

18 MG-63 cells using siRNAs targeting CD109. (b) Relative densitometric intensities of immunoblot bands
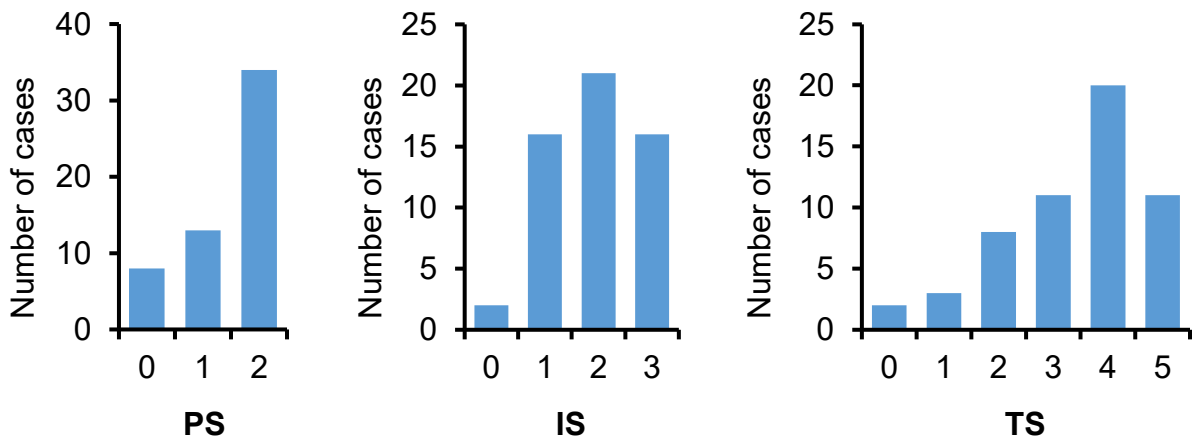
1   for ERK1/2 phosphorylation. (c) Time course of STAT3 phosphorylation after IL-6 stimulation of

2   CD109 knockdown and control MG-63 cells. (d) Time course of STAT3 phosphorylation in CD109

3   knockdown and control MG-63 cells after TGF-β stimulation. (e) Relative densitometric intensities of

4   immunoblot bands for STAT3 phosphorylation induced by IL-6 (left) or TGF-β (right). siControl,

5   Control siRNA; siCD109, siRNA targeting CD109.
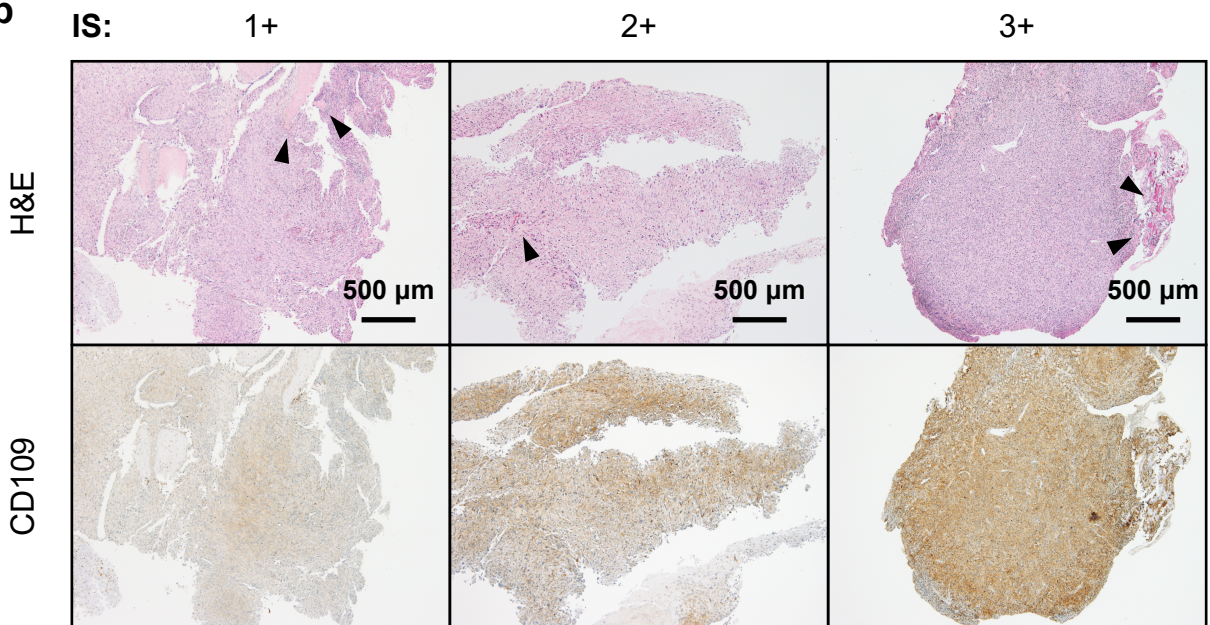
6

7   **Supplementary Fig. S4**   CD109 does not promote cell migration of human osteosarcoma cells
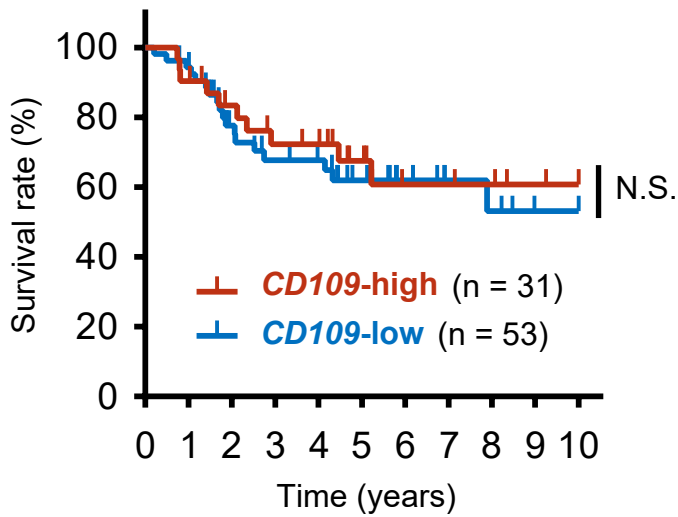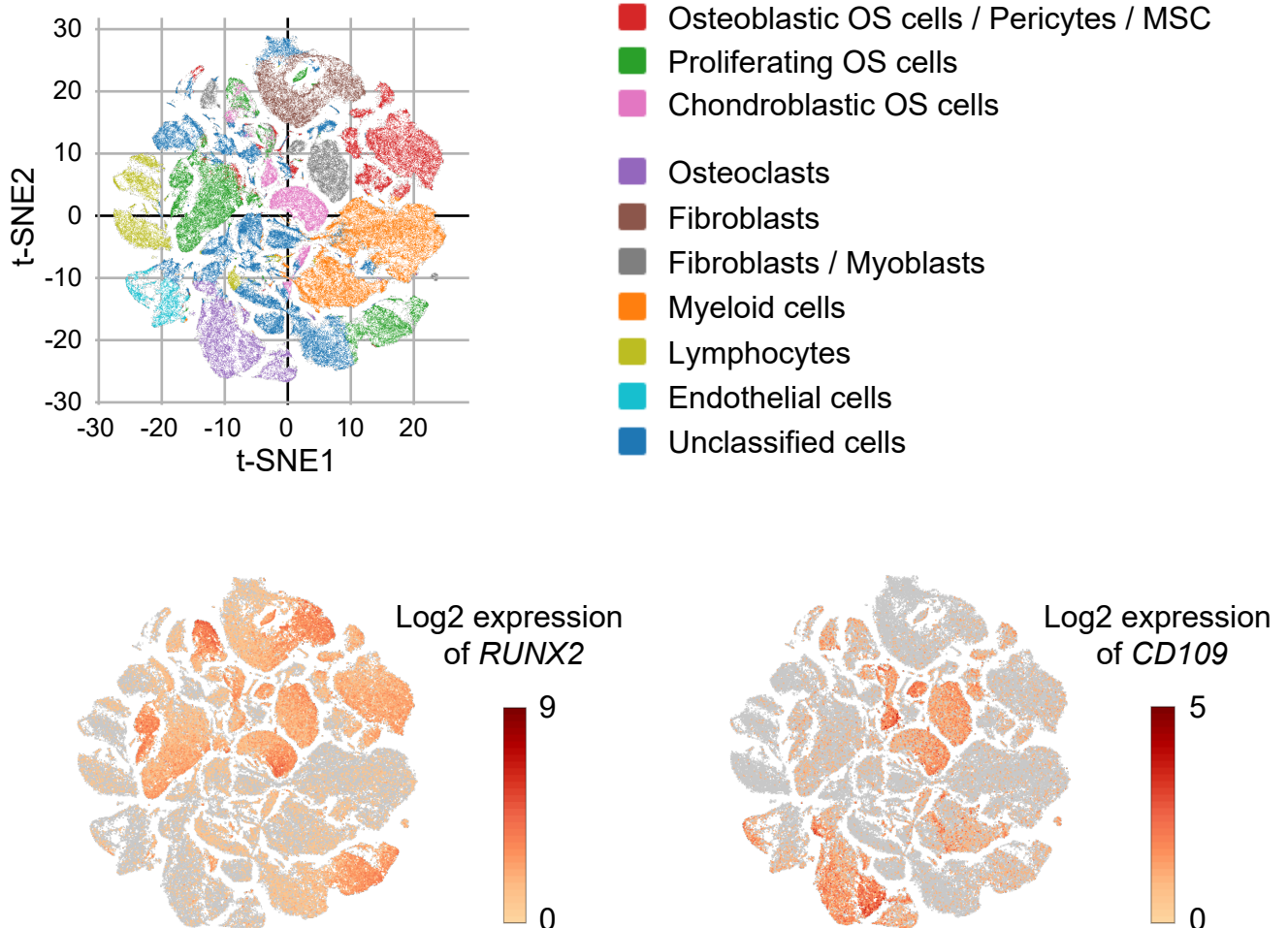
8   without the addition of BMP-2.

9   (a) Representative images of *in vitro* wound healing assays in CD109-knockdown and control MG-63

10   cells without the addition of BMP-2 (n = 3 per group). Bottom panels show images taken at 24 h after

11   wound creation. Dotted lines indicate the edge of the wound area. (b) Percentage of the unfilled wound

12   area at each time point (6, 12 and 24 h after wound creation) was calculated as described in the

13   Materials and Methods section. Error bars indicate standard deviation. siControl, Control siRNA;

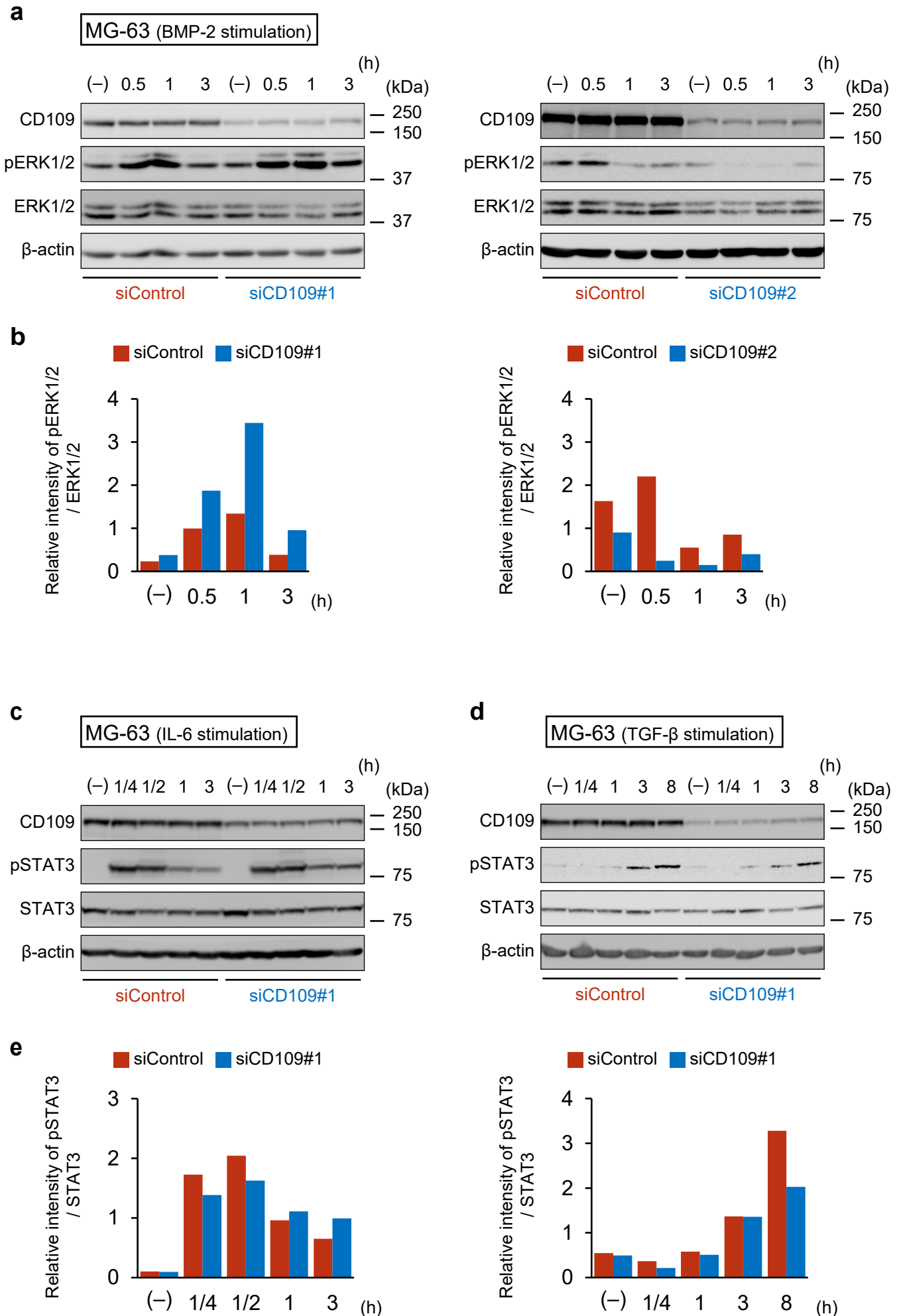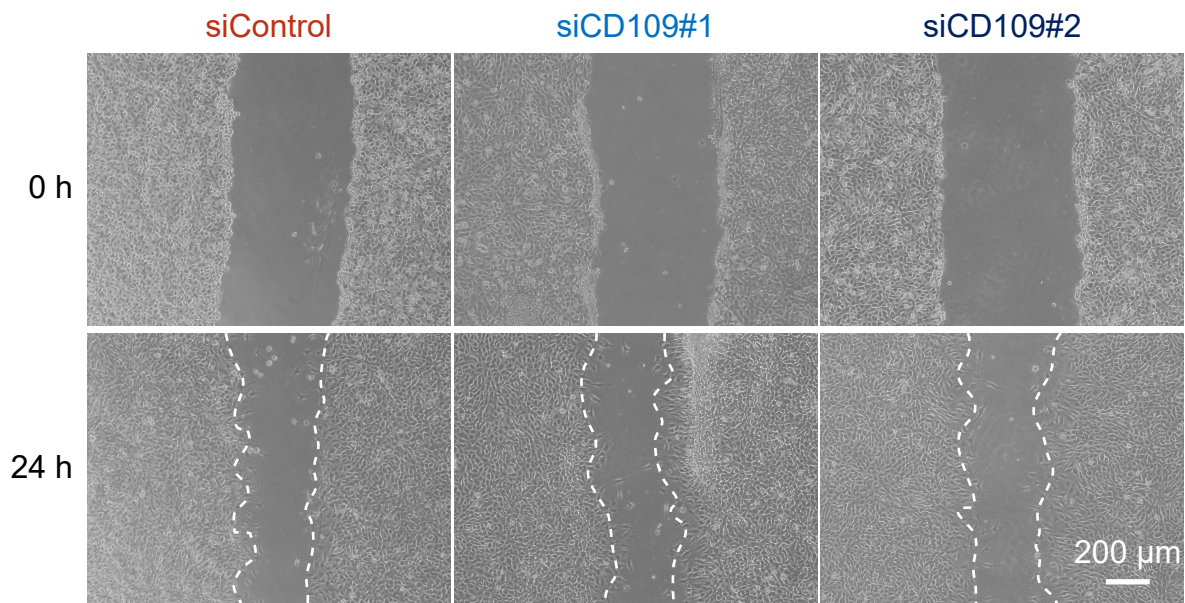14   siCD109, siRNA targeting CD109; N.S., not significant.

a

b

- Osteoblastic OS cells / Pericytes / MSC
- Proliferating OS cells
- Chondroblastic OS cells

- Osteoclasts
- Fibroblasts
- Fibroblasts / Myoblasts
- Myeloid cells
- Lymphocytes
- Endothelial cells
- Unclassified cells

Log2 expression of *RUNX2*

Log2 expression of *CD109*

a

b