SUPPLEMENTARY APPENDIX

GATA2 regulates differentiation of bone marrow-derived mesenchymal stem cells

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 doi:10.3324/haematol.2014.105692
Manuscript received on February 12, 2014. Manuscript accepted on August 7, 2014.
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Supplementary Methods

Antibodies

Allophycocyanin (APC)-labeled human CD14, phycoerythrin (PE)-labeled human CD29, PE-labeled human CD34, fluorescein isothiocyanate (FITC)-labeled mouse/human CD44, FITC-labeled human CD45, FITC-labeled human CD90 and FITC-labeled mouse Sca-1 antibodies were purchased from BD Biosciences. APC-labeled anti-human CD105, PE-labeled mouse CD29, FITC-labeled mouse CD11b, FITC labeled mouse CD34 and PE-labeled mouse CD45 antibodies were purchased from eBioscience.

Cytokine treatment

BM-MSCs were differentiated into adipocyte-lineage cells, with the presence of cytokines as follows: 100 ng/mL each of recombinant human bone morphogenetic protein 4 (rhBMP4), human transforming growth factor-β1 (rhTGFβ1), human interleukin (IL)-1β/IL-IF2z (rhIL-1β), human IL-6 (rhIL-6), human interferon-γ (rhINFγ), human tumor necrosis factor (TNF)-α (rhTNFα), or human IL-17A (rhIL-17A). All cytokines were obtained from R&D Systems.

Cell cycle fractionation

In a 6-well plate, 8.0 x 10^4 human BM-MSCs were seeded and cultured for 24 hours. On the next day, control or anti-GATA2 siRNAs were transfected. After 48 hours, the cells were collected and stained in a nucleic acid staining solution (NASS; 0.1 M phosphate-citrate buffer, pH 4.8 with 0.9 % NaCl, 0.5 % bovine serum albumin, 0.02 % saponin) supplemented with 1 μM/mL 7-aminoactinomycin D (7-AAD, Imgenez, SanDiego, CA) for 2o minutes. The cells were washed with PBS and were further stained in the NASS supplemented with 10 ng/mL pyronin Y (Sigma Aldrich, St. Louis, MO) for 5 minutes at 4℃. The cell cycle fractions were identified and quantified using FACSArisII cell sorter and BD FACSDiva software (BD Biosciences, San Jose, CA). Cells in G0 and G1 phases were determined as 7-AAD^{lo}/pyronin Y^{lo} and 7-AAD^{lo}/pyronin Y$^{int/hi}$, respectively.

Supplementary Figure Legends

Supplementary Figure 1. Phenotyping of mouse BM-MSCs.

(A) BM-MSCs were induced with iCre to delete the DNA binding domain of GATA2 using the Cre-loxP system. (B) Differentiation of $GATA2^{fl}$ BM-MSCs. Typical adipocytes contained oil drops that were stained with Oil Red O. (C) BM-MSCs from $GATA2^{fl}$ mice expressed cell-surface antigen markers for BM-MSCs. The data was a representative of 2 independent BM-MSC lines.

Supplementary Figure 2. Phenotyping of human BM-MSCs.

(A) Differentiation of human BM-MSCs. Typical adipocytes contained oil drops that were stained with Oil Red O. (B) Human BM-MSCs expressed cell-surface antigens, characteristic of BM-MSCs. The data was a representative of 3 independent BM-MSC lines.

Supplementary Figure 3. Quantitative RT-PCR-based validation analysis for *GATA2* knockdown.

The siRNA-mediated *GATA2* suppression was confirmed using quantitative RT-PCR.

The expression of *GATA2* relative to that of *GAPDH* was calculated (expressed as mean +/- SD, n = 3). Asterisk, $p < 0.05$.

Supplementary Figure 4. Increase of G1 phase cells with decreased GATA2 expression in BM-MSCs.

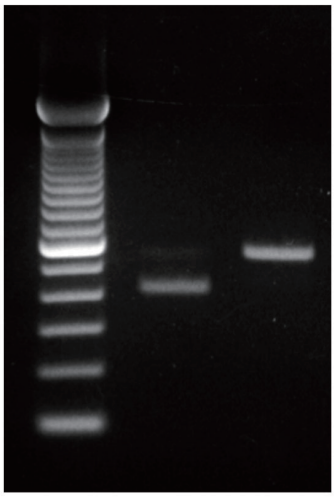
(A) Representative cell-cycle profile in human BM-MSCs treated with control or anti-GATA-2 siRNA, based on 7-AAD and pyronin Y staining. (D) Percentage of cells in G0 and G1 stages of the cell cycle (expressed as mean +/- SD, n = 3). The average percentages of G0 and G1 phase cells were significantly decreased and increased, respectively, by GATA2 knockdown ($p < 0.05$).

Supplementary Figure 5. Effects of various cytokines on adipocyte differentiation and GATA2 expression in BM-MSCs.

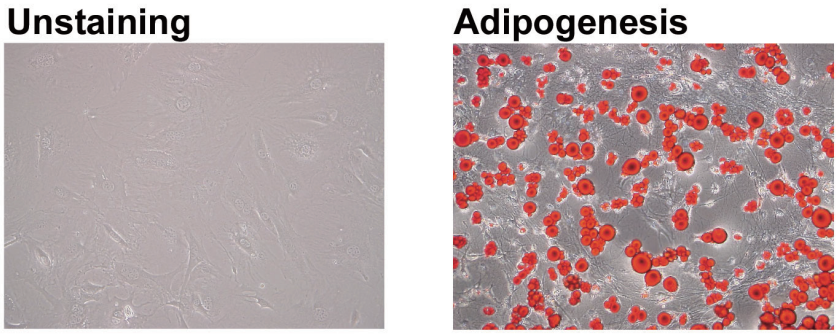
Human BM-MSCs were cultured with various cytokines (final concentration, 100 ng/mL) for 16 days, and samples were harvested on days 0, 4, 8, 12, and 16. Oil-drop formation and the expression of *GATA2* and *aP2* were analyzed using quantitative RT-PCR. (A) rhTNFα, (B) rhINFγ, (C) rhIL-1β, (D) rhIL-6, (E) IL-17A, (F) rhTGFβ1, (G)

rhBMP4. Data was expressed as mean +/- SD (n = 3). Asterisk, $p < 0.05$.

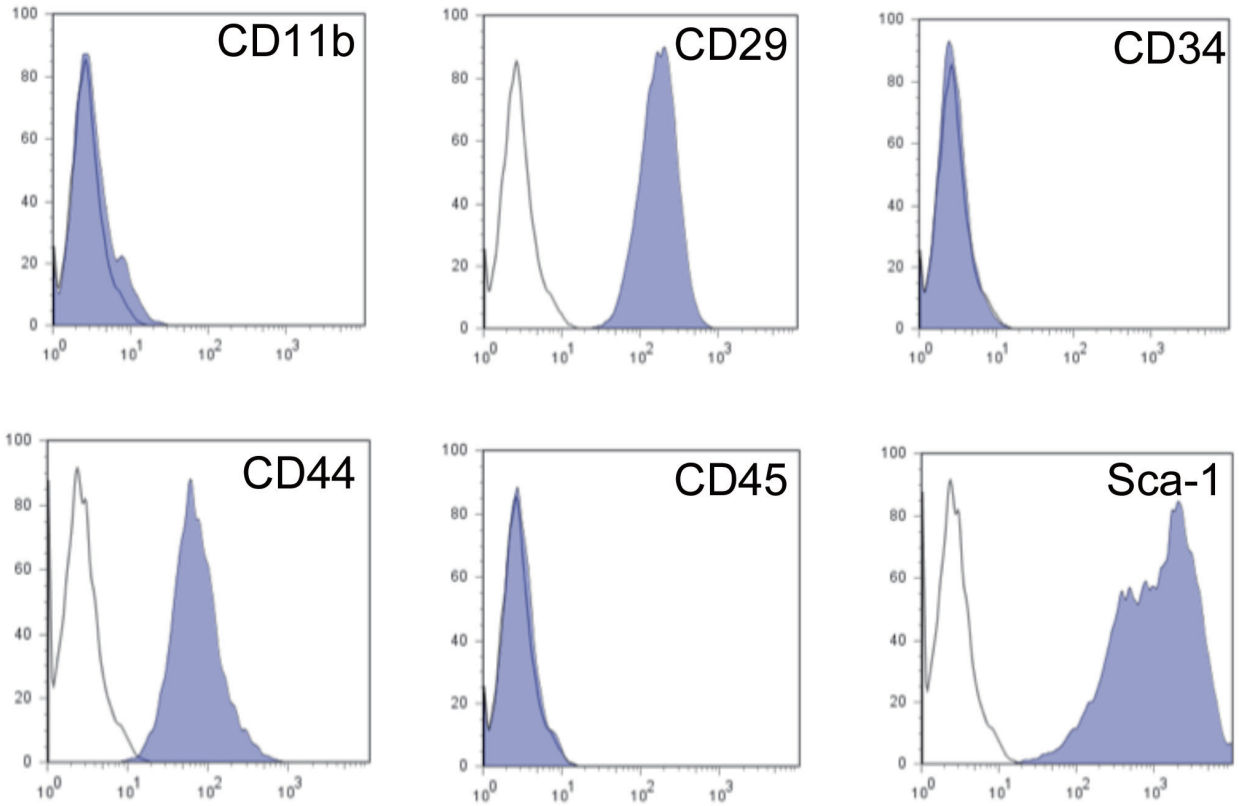
A


←GATA2fl
←GATA2-

B

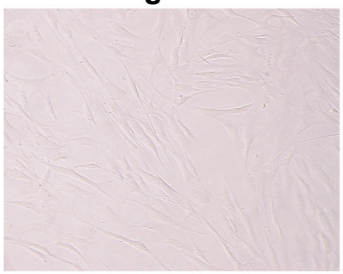

Unstaining
Adipogenesis

C

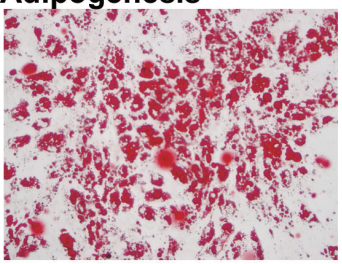

CD11b
CD29
CD34
CD44
CD45
Sca-1

A

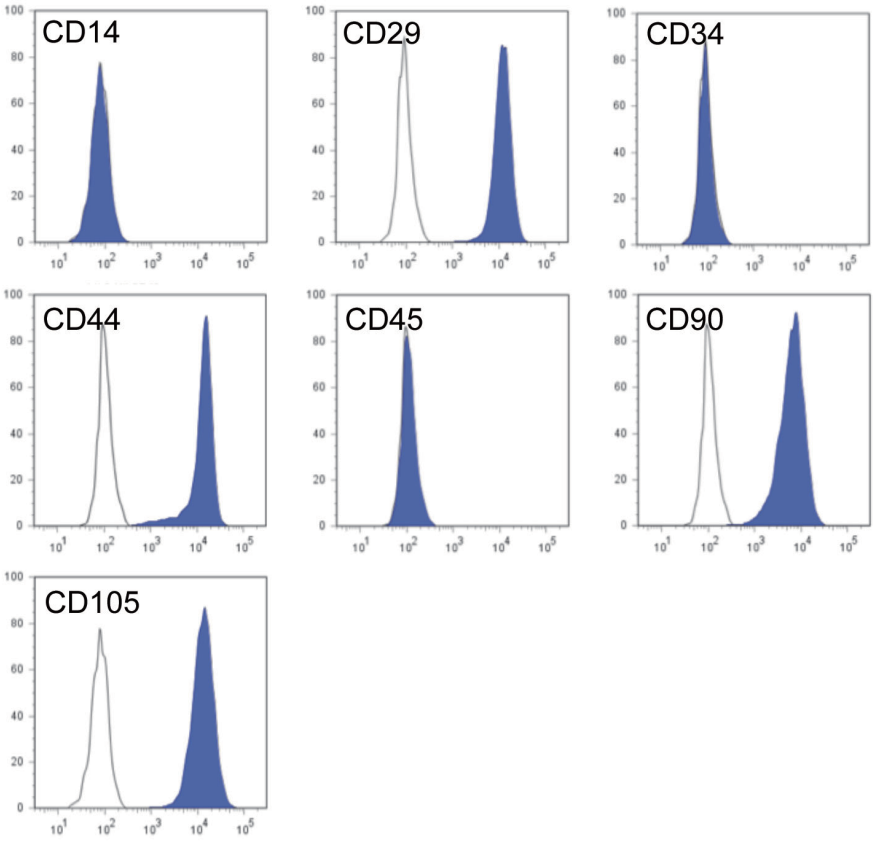
Unstaining

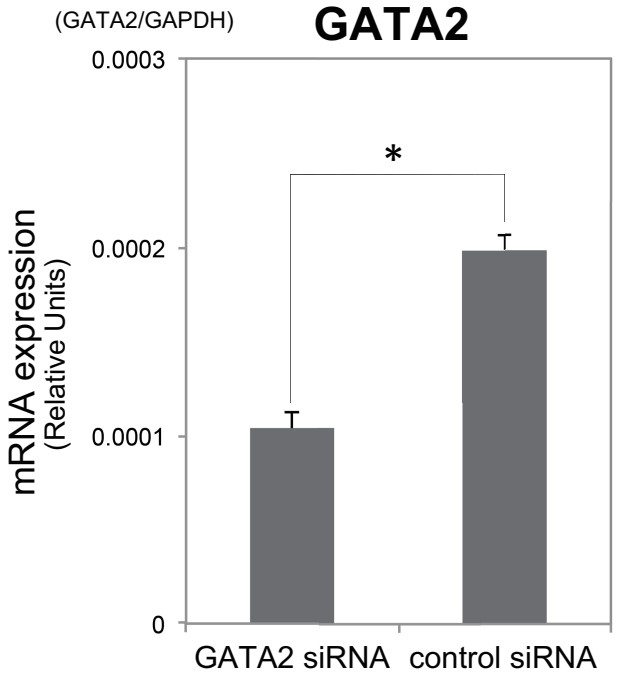


Adipogenesis

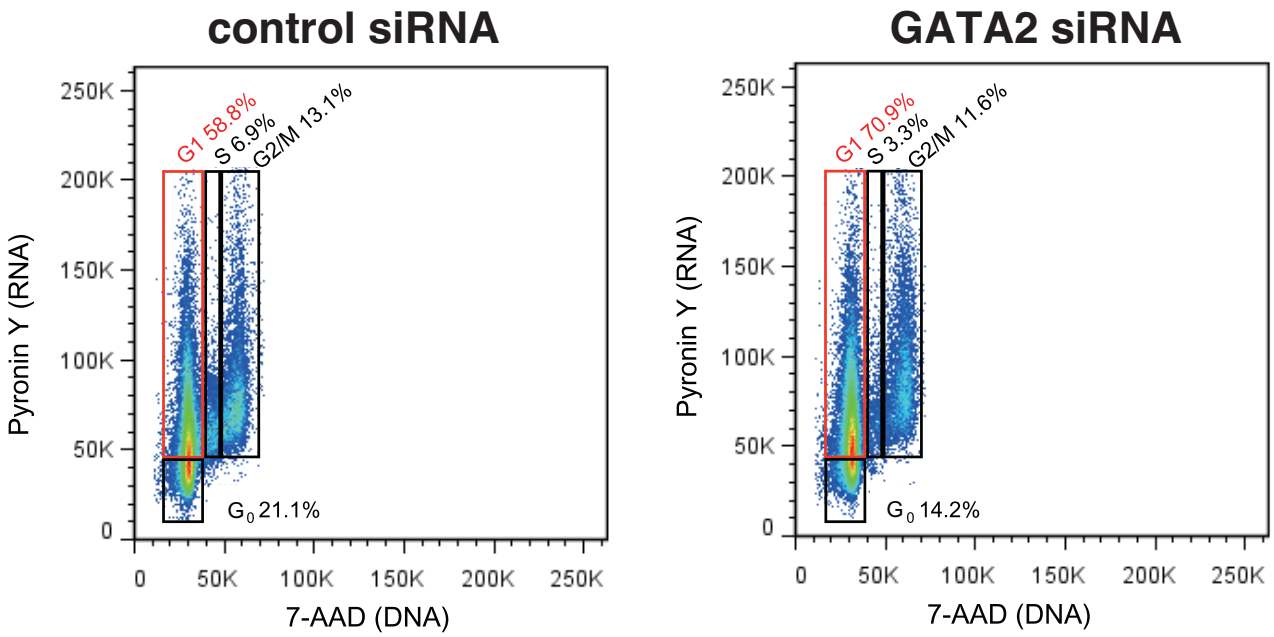


B



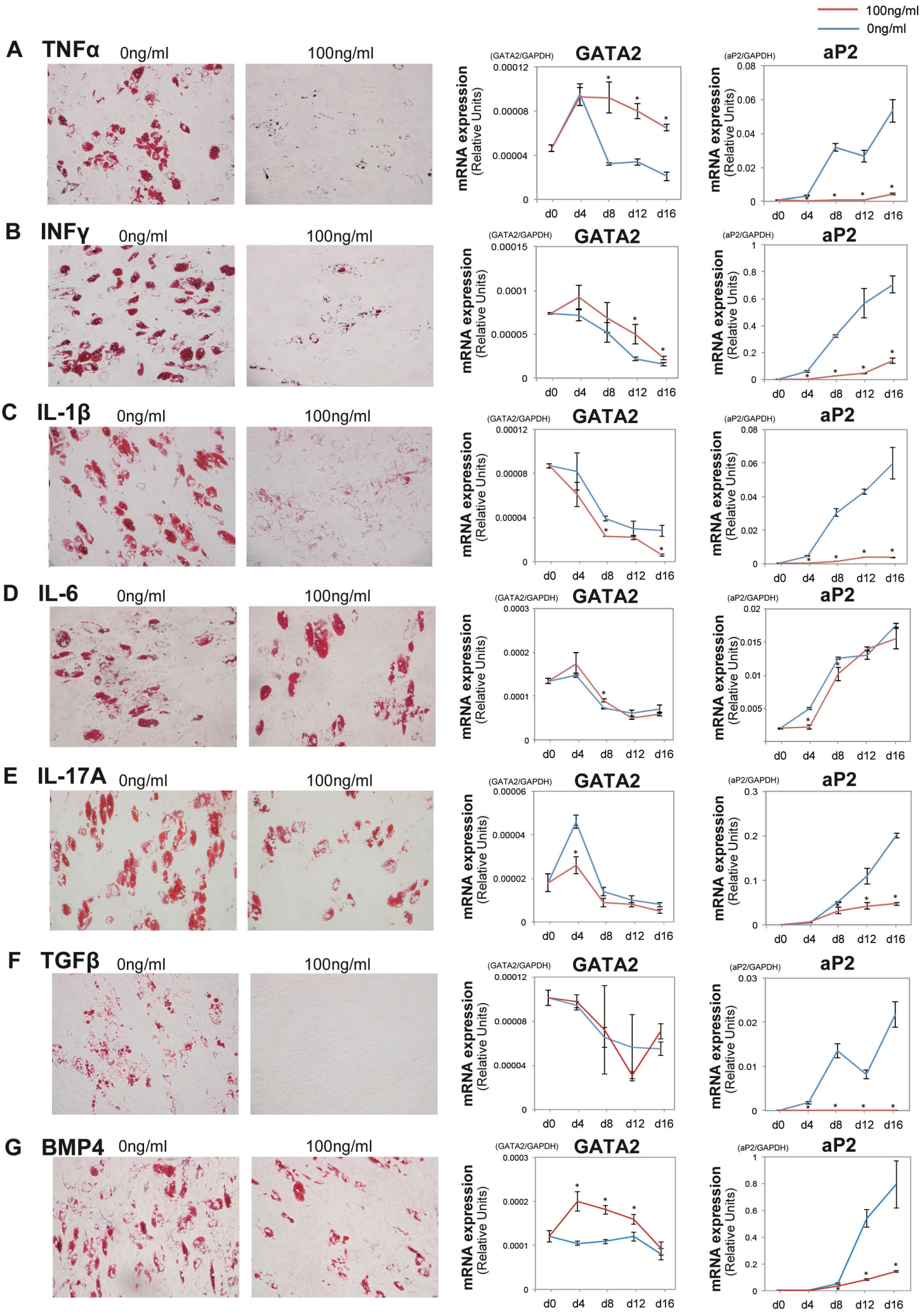

(GATA2/GAPDH)
GATA2
0.0003
0.0002
0.0001
0
*
mRNA expression
(Relative Units)
GATA2 siRNA
control siRNA

A



B

	G0 stage (%)	G1 stage (%)
control siRNA	23.0 ± 4.0	57.7 ± 3.5
GATA2 siRNA	15.1 ± 1.4	69.9 ± 1.7


100ng/ml
0ng/ml
A TNFα 0ng/ml 100ng/ml
B INFγ 0ng/ml 100ng/ml
C IL-1β 0ng/ml 100ng/ml
D IL-6 0ng/ml 100ng/ml
E IL-17A 0ng/ml 100ng/ml
F TGFβ 0ng/ml 100ng/ml
G BMP4 0ng/ml 100ng/ml
GATA2
aP2
(GATA2/GAPDH)
(aP2/GAPDH)
mRNA expression
(Relative Units)
d0 d4 d8 d12 d16