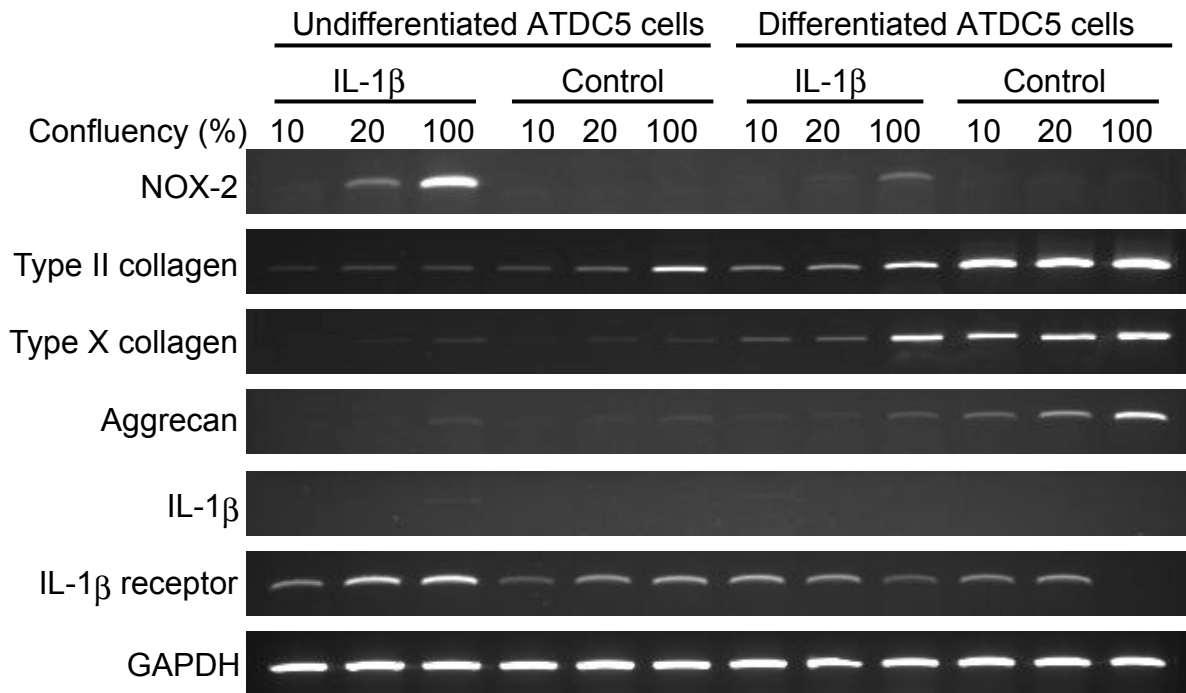


## SUPPLEMENTAL FIGURE LEGENDS

**FIGURE S1. Expression of mRNAs for IL-1 $\beta$ , IL-1 receptor, NOX-2, type II collagen, type X collagen, and aggrecan in undifferentiated and differentiated ATDC5 cells at various cell densities.** ATDC5 cells maintained in undifferentiated state and those differentiated by 2 weeks of treatment with ITS supplements were seeded into 6-well plates to reach 100% ( $2 \times 10^6$  cells/well), 20% ( $4 \times 10^5$  cells/well), or 10% ( $2 \times 10^5$  cells/well) confluence. Cells were cultured for 48 hours in the presence or absence of IL-1 $\beta$  (10 ng/ml). Total RNA was applied to analyses of mRNA expressions of NOX-2, type II collagen, type X collagen, and aggrecan by RT-PCR. The sequences of the primers used and the amplicon sizes of the genes are as follows: NOX-2, 5'-GAG GGT TTC CAG CCA GCG AAC TTT GGT-3' and 5'-TGA AGG GGG CCT GTA TGT GG-3' (349 bp); type II collagen, 5'-GCC AAG ACC TGA AAC TCT GC-3' and 5'-CTT GCC CCA CTT ACC AGT GT-3' (653 bp); type X collagen, 5'-CCA CCT GGG TTA GAT GGA AAA-3' and 5'-AAT CTC ATC AAA TGG GAT GGG-3' (583 bp); aggrecan, 5'-CAG GTT TCC CCA CTG TGT CT-3' and 5'-ACT CCA GAC CCT GGG AAG TT-3' (487 bp); IL-1 $\beta$ , 5'-GGG CCT CAA AGG AAA GAA TC-3' and 5'-CTC AGT GCA GGC TAT GAC CA-3' (612 bp); IL-1 receptor, 5'-GGG CTC ATT TGT CTC ATG GT-3' and 5'-TGTGCTCTTCAGCCACATTC-3' (556 bp); GAPDH, 5'-ACC ACA GTC CAT GCC ATC AC-3' and 5'-TCC ACC ACC CTG TTG CTG TA-3' (452 bp).

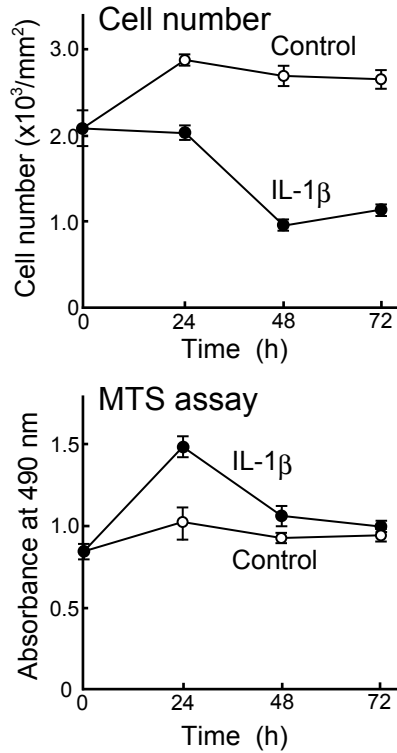
**FIGURE S2. Effects of cell density and differentiation state on IL-1 $\beta$ -induced change in viability of ATDC5 cells.** ATDC5 cells maintained in undifferentiated state (A, C) and those differentiated by 2 weeks of treatment with ITS (B, D) were seeded into 96-well plates to reach 100% ( $6 \times 10^4$  cells/well) (A, B) or 10% ( $6 \times 10^3$  cells/well) (C, D) confluence. Cells were cultured for the indicated periods in the presence or absence of IL-1 $\beta$  (10 ng/ml). The number of cells was counted after staining with toluidine blue (Cell number). Cells were incubated for 1 hour with MTS, then formed MTS-formazan was determined by reading absorbance at 490 nm (MTS assay). Data are expressed as the mean  $\pm$  S.D. of 4 independent experiments.

# Fig. S1 Yoshimura K, *et al.*

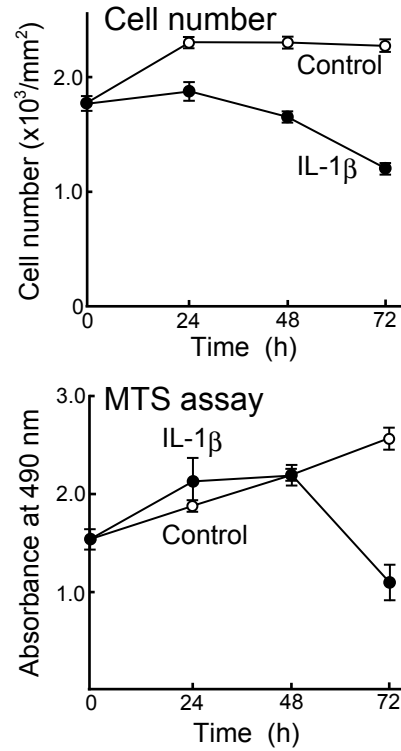


# Fig. S2 Yoshimura K, *et al.*

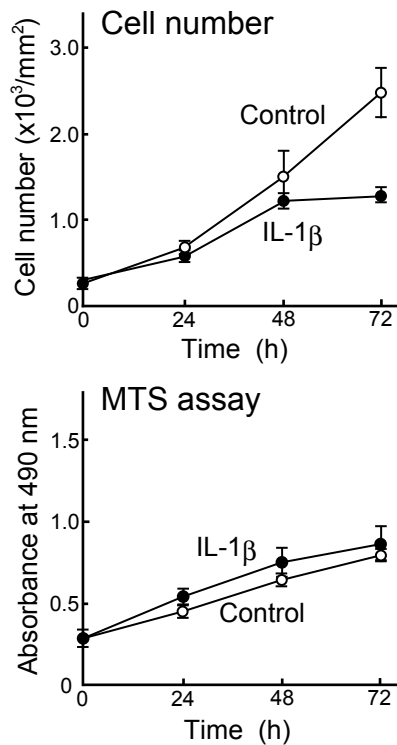
## A Undifferentiated ATDC5 cells (High density)



## B Differentiated ATDC5 cells (High density)



## C Undifferentiated ATDC5 cells (Low density)



## D Differentiated ATDC5 cells (Low density)

