Translin modulates mesenchymal cell proliferation and differentiation in mice

Ikeuchi, Yukiko ; Imanishi, Azusa ; Sudo, Katsuko ; Fukunaga, Takako ; Yokoi, Aya ; Matsubara, Leo ; Goto, Chie ; Fukuoka, Tomoya ; Kuronuma,…

(Citation)
Biochemical and Biophysical Research Communications,504(1):115-122

(Issue Date)
2018-09-26

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90005303

Highlights

1. Translin restricts the size of bone marrow mesenchymal stem cells.

2. Translin impedes the growth of early-stage mesenchymal cells.

3. Translin inhibits differentiation of bone marrow and adipose mesenchymal cells.

4. *In vitro*-fertilized Translin-null mice grow normally and show normal metabolism.

Translin modulates mesenchymal cell proliferation and differentiation in mice

Yukiko Ikeuchi [a], Azusa Imanishi [a], Katsuko Sudo [b,c], Takako Fukunaga [a], Aya Yokoi [a], Leo Matsubara [a], Chie Goto [a], Tomoya Fukuoka [a], Kana Kuronuma [a], Ruri Kono [a], Natsumi Hasegawa [a], Shigetaka Asano [c], Mitsuhiro Ito [a,c,*]

[a] Division of Medical Biophysics, Kobe University Graduate School of Health Sciences, Kobe 654-0142, Japan.

[b] Pre-clinical Research Center, Tokyo Medical University, Tokyo, 160-8402, Japan

[c] Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Tokyo 169-8555, Japan.

* Corresponding author.

TEL: +81 78 796 4546

FAX: +81 78 796 4509

E-mail address: itomi@med.kobe-u.ac.jp

Abstract

Translin, a highly conserved DNA/RNA binding protein that forms a hetero-octamer together with Translin-associated factor X (TRAX), possesses a broad variety of functions, including RNA processing and DNA repair. Recent studies have reported that Translin is involved in mesenchymal cell physiology. Thus, here we analyzed the intrinsic role of Translin in mesenchymal cell proliferation and differentiation. Translin-deficient E11.5 mouse embryonic fibroblasts showed enhanced growth. Translin-deficient bone marrow-derived mesenchymal stem cells showed substantial expansion *in vivo* and enhanced proliferation *in vitro*. These cells also showed enhanced osteogenic and adipocytic differentiation. Histological analyses showed adipocytic hypertrophy in various adipose tissues. *Translin* knockout did not affect the growth of subcutaneous white adipose tissue-derived stem cells, but enhanced adipocytic differentiation was observed *in vitro*. Contrary to previous reports, *in vitro*-fertilized Translin-null mice were not runted and exhibited normal metabolic homeostasis, indicating the fragility of these mice to environmental conditions. Together, these data suggest that Translin plays an intrinsic role in restricting mesenchymal cell proliferation and differentiation.

Key words

Translin/TRAX complex, Mesenchymal stem cells, CFU-F, Adipose-derived stem cells, Cell growth, Cell differentiation

1. Introduction

Translin, a nuclear protein that is phylogenetically conserved in eukaryotes, constitutes a hetero-octamer together with Translin-associated factor X (TRAX) [reviewed in 1, 2]. Translin was originally discovered as a protein that binds to consensus DNA sequences at breakpoint junctions of chromosomal translocations in lymphoid neoplasms and was postulated to have a biological role in genome stability [3]. Indeed, the role for Translin/TRAX complex in DNA repair especially in relation with the action of ATM, a major component for double-stranded DNA repair, is now highlighted [4, reviewed in 1, 2]. On the other hand, elucidation of the structural biology of the Translin/TRAX complex has led to the discovery of its greater affinity for single-stranded RNA/DNA and its endoribonuclease activity, which mediates processing of microRNAs and tRNAs [5-7]. However, the biological significance of Translin has not been fully documented.

Genetic ablation of murine *Translin* has allowed us to uncover the role Translin plays in a variety of biological phenomena, including cell growth [8], neurological development [9], and spermatogenesis [10]. Translin's role in mesenchymal cell physiology is of particular interest; *Translin* knockout (KO) mice have previous been shown to exhibit obesity (suggesting adipocytic hypertrophy), proliferation of osteoblasts, endothelial

cells, adipocytes, and osteocytes in bones and bone marrow (BM), altered endochondral ossification of long bones, and proliferation of fibroblasts in the liver [1, 11, 12]. All of these phenotypes appear to be related to mesenchymal cell dysfunction, indicating that Translin plays a pivotal role in mesenchyme physiology. However, it remains unknown whether they reflect Translin's intrinsic activities at a cellular level.

In order to define Translin's inherent role in mesenchymal cell proliferation and differentiation, we conducted cell biology analyses by using mice-derived mesenchymal cells. Additionally, we re-defined the runt phenotype previously found in *Translin* KO mice [13], using *in vitro*-fertilized mice. We propose that Translin is a physiological restrictor of early-stage mesenchymal cell proliferation and differentiation and provides protection from the environment.

2. Materials and methods

2-1. Mice

Translin KO mice, backcrossed at least 10 times with C57BL6 [11], were kindly provided by Masataka Kasai. For genotyping, crude genomic DNA was used for PCR analysis (primer sequences available upon request). Male mice were analyzed unless otherwise specified. All animal experiments were performed according to the institutional guidelines set forth by Tokyo Medical University, and Kobe BM Laboratory, Oriental Bioservice, Inc.

For *in vitro*-fertilized mice, two-cell embryos obtained by *in vitro* fertilization of *Translin*$^{-/-}$ sperms and *Translin*$^{+/-}$ ova were transferred into pseudo-pregnant ICR mice (Sankyo Labo Service, Tokyo, Japan). Pups were housed with their individual surrogate mothers until weaning had been achieved.

2-2. Cell culture and colony-forming units fibroblast (CFU-Fs)

Mouse embryonic fibroblasts (MEFs) were prepared from E11.5 embryos that were obtained via heterozygous crosses, and were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). MEFs at passage

2 were used for studies.

BM-derived CFU-Fs were obtained by cultures of BM cells (1.0×10^6 per 6-well plates) derived from femora and tibiae of 39-week-old males in MesenCult MSC Basal Medium supplemented with MesenCult Mesenchymal Stem Cell Stimulatory Supplements (StemCell Technologies). After 13 days of culture, May-Grünwald Giemsa staining was performed and CFU-Fs were counted. For BM-MSCs, BM-derived CFU-Fs were trypsinized, harvested and cultured in DMEM with 10% FBS.

For adipose-derived stem cells (ADSCs), subcutaneous white adipose tissues (WATs) were minced and agitated in DMEM with 0.04% collagenase (FUJIFILM Wako) for 1 h at 37°C**,** after which sediment was removed. Precipitated ADSCs were then cultured in MEM α, GlutaMAX™ Supplement, no nucleosides (Life Technologies), with 10% FBS.

2-3. Cell growth and DNA synthesis

For cell growth, cells (2×10^3 or 2×10^4) were plated on 24-well plates and counted after trypsinization. For DNA synthesis, bromodeoxyuridine (BrdU) was added to the cells on 24-well plates. After purging for 6 h, the incorporation of BrdU was measured

as previously described [14].

2-4. Osteogenic differentiation of BM-MSCs

When cells reached 30-50% confluency, osteogenic differentiation was induced by exchanging the media for StemXVivo Human/Mouse Osteogenic Base Media (R&D Systems) supplemented with 5% StemXVivo Mouse Osteogenic Supplement (R&D Systems) by volume. After induction, cells were subjected to staining or extraction of total RNA with Isogen II (Toyobo) and quantification. Staining was performed with one of two stains; for alkaline phosphatase, cells were fixed with 4% paraformaldehyde for 15 min and stained with VECTOR Red Alkaline Phosphatase Substrate Kit (Vector Laboratories) according to the manufacturer's protocol. For Alizarin Red S, cells were fixed in 95% ethanol, washed with H_2O, and stained with 1% Alizarin Red S (FUJIFILM Wako) for 30 min.

2-5. Adipocytic differentiation of BM-MSCs and ADSCs

Adipocytic differentiation was induced using AdipoInducer Reagent (TaKaRa) according to the manufacturer's protocol when cells reached 80-90% confluency. Medium was exchanged twice a week. After induction, the cells were either stained for

lipid droplets with Oil Red O (FUJIFILM Wako), or total RNA was extracted. After Oil Red O staining, the dye was extracted by incubating cells with isopropanol for 30 min, and quantified by measuring the optical density at 450 nm (OD_{450}).

2-6. Histological analysis

Tissues were stored in 10% neutral formalin and embedded in paraffin blocks; each section was deparaffinized, rehydrated, and stained with hematoxylin and eosin.

2-7. Quantitative RT-PCR

Total cellular RNA (0.5 μg) was reverse-transcribed with ReverTra Ace qPCR RT Master Mix with a gDNA Remover kit (Toyobo). Quantitative PCR (StepOnePlus Real-Time PCR system; Thermo Fisher Scientific) was performed for quantification of mRNA. Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize results. The sequences of the primers used in amplification are available upon request.

2-8. Statistical analyses

All numerical results were expressed as means ± SD. Data were compared using Student's t-test (two groups) or one-way analysis of variance (ANOVA) (more than two groups). Two-way ANOVA was used for studies over a prolonged time and, when significant, values for each time point were evaluated by Student's t-test or one-way ANOVA. We considered $P < 0.05$ as significant. $P < 0.05$ and $P < 0.01$ were represented by * and **, respectively.

3. Results

3-1. Translin restricts growth of MEFs

In previous studies, *Translin*$^{-/-}$ mice were reported to be smaller [13] and *Translin*$^{-/-}$ E14.5 MEFs were found to grow more slowly [8] than *Translin*$^{+/+}$ and *Translin*$^{+/-}$ controls, suggesting that Translin intrinsically promotes cell growth. By contrast, in another study, *Translin*$^{-/-}$ mice displayed obesity in addition to increased numbers of osteoblasts and osteocytes, adipocytes, endothelial cells, and fibroblasts in aged *Translin*$^{-/-}$ bones and liver [11], indicating that Translin restricts mesenchymal cell growth. To resolve these discrepancies, we hypothesized that Translin restricts the growth and differentiation solely of immature mesenchymal cells. To test this hypothesis, we studied the effects of *Translin* KO on MEFs of a more primitive stage, namely, E11.5.

Indeed, contrary to the slow growth of *Translin*$^{-/-}$ E14.5 MEFs [8], the growth rates of *Translin*$^{+/+}$, *Translin*$^{+/-}$ and *Translin*$^{-/-}$ E11.5 MEFs were inversely proportional to *Translin* levels (Fig. 1A). The BrdU incorporation assay to measure MEF mitogenicity revealed enhanced DNA synthesis in *Translin*$^{-/-}$ and *Translin*$^{+/-}$ MEFs compared with that in *Translin*$^{+/+}$ MEFs (Fig. 1B). These results indicated that Translin specifically

restricts early-stage mesenchymal precursor cells.

3-2. Translin restricts growth of BM-MSCs

We next explored Translin's activity in adult immature mesenchymal cells using BM-MSCs, which possess multilineage differentiation potential and have been studied extensively in relation to hematopoiesis [reviewed in 15]. We selected slightly old (39-week-old) mice; at this age, *Translin*$^{-/-}$, *Translin*$^{+/-}$ and *Translin*$^{+/+}$ BMs were histologically indistinguishable and complete blood counts were comparable (data not shown). CFU-Fs, which arise from MSCs, developed when BM cells were cultured in media that preferentially supported growth of MSCs. The number of *Translin* KO CFU-Fs was inversely proportional to *Translin* levels (Fig. 1C). The number of CFU-Fs is representative of the *in vivo* size of MSCs; therefore, these results imply that *Translin* KO mice possess more BM-MSCs than wild-type control mice.

We asked if the growth of *Translin* KO BM-MSCs was intrinsically enhanced, and next explored the growth rate of these cells by counting the MSCs after the second passage of CFU-F trypsinization. The number of *Translin*$^{-/-}$ BM-MSCs was greater than that of the wild-type controls, although the number of dead cells were comparable (Fig. 1D).

DNA synthesis was also enhanced in *Translin*$^{-/-}$ BM-MSCs relative to that in wild-type BM-MSCs (Fig. 1D), indicating that *Translin*$^{-/-}$ BM-MSCs grow faster than wild-type BM-MSCs. Similarly, the number and mitogenicity of *Translin*$^{+/-}$ BM-MSCs were higher than those of the wild-type controls (Supplementary Fig. 1). However, the numbers of both living and dead *Translin*$^{+/-}$ BM-MSCs were the same as those of *Translin*$^{-/-}$ BM-MSCs, and DNA synthesis in these BM-MSCs was comparable (Fig. 1E). These results suggest that the increased growth of *Translin* KO BM-MSCs is both intrinsic and haplosufficient and that Translin restricts the number of BM-MSCs *in vivo*.

3-3. Translin restricts osteogenic differentiation of BM-MSCs

A previous study reported increased numbers of osteoblasts and adipocytes in older *Translin*$^{-/-}$ mice [11]. Thus, we next determined if the differentiation ability of *Translin* KO BM-MSCs was intrinsically affected. First, we measured their capacity for osteogenic differentiation via cytochemical analyses of both early-stage (alkaline phosphatase staining) and late-stage (alizarin red S staining) differentiation markers and quantifying the expression of marker genes for early-stage (*Runx2*) and late-stage (gene encoding collagen type 1α1; *Col1α1*) differentiation.

At 14 days after osteogenic induction of BM-MSCs, both *Translin*$^{-/-}$ and *Translin*$^{+/-}$ BM-MSCs showed enhanced staining for alkaline phosphatase (Fig. 2A). At this stage of differentiation, *Translin*$^{-/-}$ BM-MSCs showed greater *Runx2* transcript levels than *Translin*$^{+/+}$ BM-MSCs did (Fig. 2B). After 21 days, staining for alkaline phosphatase was prominently strengthened in *Translin*$^{+/+}$ BM-MSCs, but already passed a peak of their strength in *Translin*$^{-/-}$ and *Translin*$^{+/-}$ BM-MSCs (Fig. 2A). However, alizarin red S staining revealed greater calcium deposition in *Translin*$^{-/-}$ and *Translin*$^{+/-}$ BM-MSCs than in *Translin*$^{+/+}$ BM-MSCs (Fig. 2C). At this stage, *Col1α1* was prominently induced in *Translin*$^{-/-}$ BM-MSCs relative to that in *Translin*$^{+/+}$ controls (Fig. 2D). These results suggest that *Translin*$^{-/-}$ BM-MSCs are intrinsically more prone to osteogenic differentiation.

3-4. Translin restricts adipocytic differentiation of BM-MSCs

Although BM-MSCs were more committed to the osteoblastic lineage, we observed small amounts of adipocytic differentiation. When differentiation was induced for 21 days, oil droplets were more pronounced in *Translin*$^{-/-}$ and *Translin*$^{+/-}$ BM-MSCs than in *Translin*$^{+/+}$ BM-MSCs (Fig. 2E). Expression of *Pparγ* and *Lpl*, marker genes for adipogenesis, was more prominently induced in *Translin*$^{-/-}$ BM-MSCs than in

Translin$^{+/+}$ controls (Fig. 2F). These data suggest that *Translin* KO BM-MSCs are intrinsically more prone to adipocytic differentiation than wild-type BM-MSCs and, together with the abovementioned observations, that Translin intrinsically restricts osteogenic and adipocytic differentiation of BM-MSCs.

3-5. Translin restricts adipocytic hypertrophy in vivo

Previous studies focusing on obesity in *Translin*$^{-/-}$ mice [16] inferred a connection with white adipocytic hypertrophy. Mammals have three distinct types of adipose tissue, namely visceral WAT, subcutaneous WAT and brown adipose tissue (BAT), all of which are thought to have distinct developmental origins, anatomical structures, and function [17, 18]. Histological analyses of 12-week-old mice revealed enhanced adipocytic hypertrophy in the visceral and subcutaneous WATs of female *Translin* KO mice (Fig. 3A, B). Increased adipocytic hypertrophy was also observed in the BATs of male *Translin*$^{-/-}$ mice (supplementary Fig. 2A, B), resulting in *Translin*$^{-/-}$ BATs being heavier than the controls (supplementary Fig. 2C). Thus, adipocytes of diverse developmental origins appear to predispose to enhanced maturation.

3-6. Translin does not affect growth of, but restricts differentiation of, ADSCs

As the adipocytic hypertrophy was most prominently observed in subcutaneous WATs of female *Translin* KO mice (Fig. 3A, B), we next determined whether the phenotype was intrinsic by assessing primary ADSC cultures. Growth and mitogenicity of *Translin* KO ADSCs harvested from subcutaneous WATs were similar to those ADSCs from wild-type controls (Fig. 3C). Considering that ADSCs may be lineage-committed to adipocytes while BM-MSCs are less differentiated and display multi-lineage behavior, the unaffected growth of *Translin* KO ADSCs is consistent with the hypothesis (above) that Translin restricts the growth of immature mesenchymal cells.

However, in Oil Red O staining after inducing of adipocytic differentiation for 21 days, the staining intensity was inversely proportional to the *Translin* levels, with *Translin*$^{-/-}$ ADSCs being the most strongly stained (Fig. 3D). Expression of adipocytic differentiation markers (*Pparγ* and *Lpl*) was also upregulated in *Translin*$^{-/-}$ ADSCs (Fig. 3E). These results collectively suggest that Translin does not affect the growth of ADSCs, but *does* restrict their differentiation.

3-7. In vitro fertilized Translin$^{-/-}$ *mice grow normally*

In one study, *Translin*$^{-/-}$ mice were reported to suffer from dwarfism, being smaller than

Translin$^{+/+}$ and *Translin*$^{+/-}$ mice [13]. However, our study suggested that Translin impedes the growth and differentiation of early mesenchymal cells that constitute the future skeleton and other various tissues. To resolve this discrepancy, we hypothesized that the runt phenotype of *Translin*$^{-/-}$ mice was not genetically determined, but was instead environmentally caused. To explore the environmental effects on embryos and pups, we performed *in vitro* fertilization of C57BL6 *Translin*$^{-/-}$ sperms and *Translin*$^{+/-}$ ova, and transferred the embryos to pseudo-pregnant ICR female mice that were individually housed.

Body sizes of *in vitro*-fertilized *Translin*$^{-/-}$ and *Translin*$^{+/-}$ pups were indistinguishable. After weaning, the weights of both male and female *Translin*$^{-/-}$ pups were the same as littermate *Translin*$^{+/-}$ mice (Fig. 4A). This result shows that the runt phenotype of naturally born *Translin*$^{-/-}$ mice is caused by the environment in which they develop, which is attributable to *Translin*$^{+/-}$ C57BL6 females. Thus, this phenotype is likely epigenetic in nature. ICR females produce more milk and are better at nursing pups than C57BL6 females [19], which suggests that *in vitro*-fertilized litters may have been raised in better environments with little competition among the pups for milk.

3-8. Basal metabolism of in vitro-fertilized Translin$^{-/-}$ mice is unaffected

As growth and adipose physiology are intricately linked to basal metabolic condition, we next explored the differences in the basal metabolism of *in vitro*-fertilized mice. Body temperatures of both male and female *in vitro*-fertilized *Translin*$^{-/-}$ mice were the same as those of littermate *Translin*$^{+/-}$ mice (Fig. 4B). Food intakes of both male and female *in vitro*-fertilized *Translin*$^{-/-}$ mice were also comparable with those of littermate *Translin*$^{+/-}$ mice (Fig. 4C). Hence, the basal metabolism of *in vitro*-fertilized *Translin*$^{-/-}$ mice appears unaltered relative to that of *Translin*$^{+/-}$ mice.

4. Discussion

Detailed analysis of mouse model-derived mesenchymal cells has revealed novel physiological roles for the phylogenetically conserved endonuclease Translin. This is the first study to identify Translin as a negative regulator of primitive mesenchymal cell proliferation and mesenchymal cell differentiation. The absence of the runting phenotype in the *in vitro*-fertilized Translin-null mice in this study further indicates that Translin protects both perinatal and postnatal growth from environmental fluctuation (Fig. 4D). These physiological roles may reflect the complex sum of nuclease activities of this protein (or Translin/TRAX complex) within mesenchymal cells.

MEFs are often used for cytogenetic studies on genes of interest. However, MEFs at different developmental stages are quite diverse. The discrepancy between a previous study [8] and the current findings may be attributable to the different developmental stages of the mesenchyme from which the MEFs were derived; E11.5 MEFs are thought to be more primitive than E14.5 MEFs. The increased growth of *Translin* KO BM-MSCs but normal proliferation of *Translin* KO ADSCs may also be explained by the fact that BM-MSCs are less lineage-committed than ADSCs.

However, both *Translin* KO BM-MSCs and ADSCs showed enhanced differentiation. This is consistent with the hypertrophied adipocytes of both *Translin* KO WATs and BATs and indicates that Translin restricts differentiation even in relatively committed mesenchymal cells. Thus, Translin may play a role in governing mesenchymal cell growth and differentiation. A cell-engineering technique wherein Translin is inhibited within cultured MSCs could be a useful strategy for expanding, and enhancing the plasticity, of these cells *ex vivo*, and may be of clinical value in the future.

An animal's health is subject to environmental factors both at intrauterine and postnatal stages, and they are marked as epigenetics. Our observation of *in vitro*-fertilized mice suggests that the previously reported runt phenotype of *Translin*$^{-/-}$ mice is not genetically derived, but is instead attributable to a poor perinatal environment. Paucity of milk and poor nursing from C57BL6 females exposes pups to competition with their litter-mates for survival. This finding may imply that Translin is required in such a severe environment. As environmental changes often bring about epigenetic alterations, epigenetics in naturally born *Translin* KO mice relative to that in *in vitro*-fertilized *Translin* KO mice should be carefully analyzed in the future. Additionally, the relation between epigenetic alterations and Translin's endonuclease activities also remains to be

studied.

In conclusion, Translin is physiologically necessary for restricting the size and differentiation of early-stage mesenchymal cells and, possibly, for the protection of these cells from epigenetic alterations caused by harsh environmental conditions. The mechanisms underlying these functions require further investigation.

Acknowledgment

We thank Isao Sato, Kiyoto Yamada, Miyuki Shimane, Hitoshi Nomura, Yoichi Nakao, Koichiro Tsuji, and members of our laboratories at Waseda and Kobe Universities for helpful discussion. This work was supported by research fund from Chugai Pharmaceutical Co. Ltd. to Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, and by Grants-in-Aid for Scientific Research (26460677 and 17K09012) from the Japan Society for the Promotion of Science.

References

[1] M. Kasai, R. Ishida, K. Nakahara, K. Okumura, K. Aoki, Mesenchymal cell differentiation and diseases: involvement of translin/TRAX complexes and associated proteins, Ann. N. Y. Acad. Sci. 1421 (2018) 37-45.

[2] Y. Chern, T. Chien, X. Fu, A.P. Shah, T. Abel, J.M. Baraban, Trax: A versatile signaling protein plays key roles in synaptic plasticity and DNA repair, Neurobiol. Learn. Mem. 2018, in press, doi: 10.1016/j.nlm.2018.07.003.

[3] K, Aoki, K. Suzuki, T. Sugano, T. Tasaka, K. Nakahara, O. Kuge, A. Omori, M. Kasai, A novel gene, Translin, encodes a recombination hotspot binding protein associated with chromosomal translocations, Nat. Genet. 10 (1995) 167-174.

[4] J.Y. Wang, S.Y. Chen, C.N. Sun, T. Chien, Y. Chern, A central role of TRAX in the ATM-mediated DNA repair, Oncogene 35 (2016) 1657-1670.

[5] Y. Liu, X. Ye, F. Jiang, C. Liang, D. Chen, J. Peng, L.N. Kinch, N.V. Grishin, Q. Liu, C3PO, an endoribonuclease that promotes RNAi by facilitating RISC activation,

Science 325 (2009) 750-753.

[6] Y. Tian, D.K. Simanshu, M. Ascano, R. Diaz-Avalos, A.Y. Park, S.A. Juranek, W.J. Rice, Q. Yin, C.V. Robinson, T. Tuschl, D.J. Patel, Multimeric assembly and biochemical characterization of the Trax-translin endonuclease complex, Nat. Struct. Mol. Biol. 18 (2011) 658-664.

[7] L. Li, W. Gu, C. Liang, Q. Liu, C.C. Mello, Y. Liu, The translin-TRAX complex (C3PO) is a ribonuclease in tRNA processing, Nat. Struct. Mol. Biol. 19 (2012) 824-830.

[8] S. Yang, Y.S. Cho, V.M. Chennathukuzhi, L.A. Underkoffler, K. Loomes, N.B. Hecht, Translin-associated factor X is post-transcriptionally regulated by its partner protein TB-RBP, and both are essential for normal cell proliferation, J. Biol. Chem. 279 (2004) 12605-12614.

[9] Z. Li, Y. Wu, J.M. Baraban, The Translin/Trax RNA binding complex: clues to function in the nervous system, Biochim. Biophys. Acta 1779 (2008) 479-485.

[10] Y.S. Cho, N. Iguchi, J. Yang, M.A. Handel, N.B. Hecht, Meiotic messenger RNA and noncoding RNA targets of the RNA-binding protein Translin (TSN) in mouse testis, Biol. Reprod. 73 (2005) 840-847.

[11] R. Ishida, K. Aoki, K. Nakahara, Y. Fukuda, M. Ohhori, Y. Saito, K. Kano, J. Matsuda, S. Asano, R.T. Maziarz, M. Kasai, Translin/TRAX deficiency affects mesenchymal differentiation programs and induces bone marrow failure, in: Srivastava R (Ed.), Stem Cells and Human Diseases, Springer, Dordrecht, 2012, pp 467-484.

[12] J.Y. Cheng, S.H. Wang, J. Lin, Y.C. Tsai, J. Yu, J.C. Wu, J.T. Hung, J.J. Lin, Y.Y. Wu, K.T. Yeh, A.L. Yu, Globo-H ceramide shed from cancer cells triggers translin-associated factor X-dependent angiogenesis, Cancer Res. 274 (2014) 6856-6866.

[13] V. Chennathukuzhi, J.M. Stein, T. Abel, S. Donlon, S. Yang, J.P. Miller, D.M. Allman, R.A. Simmons, N.B. Hecht, Mice deficient for testis-brain RNA-binding

protein exhibit a coordinate loss of TRAX, reduced fertility, altered gene expression in the brain, and behavioral changes, Mol. Cell. Biol. 23 (2003) 6419-6434.

[14] A. Sumitomo, R. Ishino, N. Urahama, K. Inoue, K. Yonezawa, N. Hasegawa, O. Horie, H. Matsuoka, T. Kondo, R.G. Roeder, M. Ito. The transcriptional mediator subunit MED1/TRAP220 in stromal cells is involved in hematopoietic stem/progenitor cell support through osteopontin expression, Mol. Cell. Biol. 30 (2010) 4818-4827.

[15] S.J. Morrison, D.T. Scadden, The bone marrow niche for haematopoietic stem cells, Nature 505 (2014) 327-334.

[16] R. Brommage, U. Desai, J.P. Revelli, D.B. Donoviel, G.K. Fontenot, C.M. Dacosta, D.D. Smith, L.L. Kirkpatrick, K.J. Coker, M.S. Donoviel, D.E. Eberhart, K.H. Holt, M.R. Kelly, W.J. Paradee, A.V. Philips, K.A. Platt, A. Suwanichkul, G.M. Hansen, A.T. Sands, B.P. Zambrowicz, D.R. Powell, High-throughput screening of mouse knockout lines identifies true lean and obese phenotypes, Obesity 16 (2008) 2362-2367.

[17] C. Hepler, L. Vishvanath, R.K. Gupta, Sorting out adipocyte precursors and their role in physiology and disease, Genes Dev. 31 (2017) 127-140.

[18] J. Chi, Z. Wu, C.H.J. Choi, L. Nguyen, S. Tegegne, S.E. Ackerman, A. Crane, F. Marchildon, M. Tessier-Lavigne, P. Cohen, Three-dimensional adipose tissue imaging reveals regional variation in beige fat biogenesis and PRDM16-dependent sympathetic neurite density, Cell Metab. 27 (2018) 226-236.

[19] G. Eaton, F. Johnson, R. Custer, A. Crane, The Icr:Ha(ICR) mouse: a current account of breeding, mutations, diseases and mortality, Lab. Anim. 14 (1980) 17-24.

Legends to Figures

Fig. 1. Translin restricts growth of mouse embryonic fibroblasts (MEFs) and bone marrow (BM)-derived mesenchymal stem cells (MSCs).

A. The growth rate of *Translin* KO MEFs, obtained from E11.5 embryos, was inversely proportional to *Translin* levels.

B. DNA synthesis of the same MEFs at day 3, measured by BrdU incorporation, was enhanced in *Translin*$^{-/-}$ and *Translin*$^{+/-}$ MEFs compared with that in *Translin*$^{+/+}$ MEFs.

C. The number of CFU-Fs, obtained from *Translin* KO BMs, was increased compared with that in wild-type controls. We considered a lump of more than 3 cells as a colony. Large- and medium-sized colonies represent lumps of over approximately 400 cells and of over approximately 200 cells, respectively. Representative colonies, stained by May-Grünwald Giemsa staining, are shown (right).

D. Cell number (left panel) and DNA synthesis (right panel) of *Translin*$^{-/-}$ BM-MSCs were enhanced compared wirh *Translin*$^{+/+}$ BM-MSCs, while cell death of *Translin*$^{-/-}$ BM-MSCs was not altered (left panel). Living (circles) and dead (triangles) cells, determined by trypan blue staining, are shown (left panel).

E. Cell number (left panel) and DNA synthesis (right panel), as well as cell death (left

panel), of *Translin*$^{-/-}$ BM-MSCs were not altered compared with *Translin*$^{+/-}$ BM-MSCs, indicating haploinsufficiency of *Translin* for growth inhibition of BM-MSCs.

N = 4 (A) or 3 (B-E).

Fig. 2. Translin restricts osteogenic and adipocytic differentiation of BM-MSCs.

A and B. Alkaline phosphatase staining (A) and *Runx2* expression (B), indicators of early-stage osteogenic differentiation, of BM-MSCs after osteogenic induction demonstrated that osteogenic commitment of *Translin* KO BM-MSCs was enhanced.

C and D. Alizarin red staining (C) and ***Col1α1*** expression (D), indicators of late-stage osteogenic differentiation, of BM-MSCs after osteogenic induction demonstrated that osteogenic differentiation of *Translin* KO BM-MSCs was enhanced.

E and F. Oil red O staining (E) and expression of *Pparγ* and *Lpl* (F), indicators of adipocytic differentiation, of BM-MSCs after adipocytic induction demonstrated that adipocytic differentiation of *Translin* KO BM-MSCs was enhanced.

N = 4 (B, D, F).

Fig. 3. Translin restricts adipocytic differentiation and hypertrophy of adipose tissues.

A and B. Adipocytes of white adipose tissues (WATs) in female *Translin* KO mice were hypertrophied. The numbers of adipocytes per unit area showed that *Translin* KO white adipocytes of both visceral (gonadal) and subcutaneous (inguinal) WATs were more hypertrophied than wild-type controls (B). Bar, 100 μm.

C. Growth (left panel) and DNA synthesis (right panel) of *Translin* KO adipose-derived stem cells (ADSCs) taken from subcutaneous (inguinal) WATs were not altered.

D and E. Oil Red O staining (D) and expression of *Pparγ* and *Lpl* (E), indicators of adipocytic differentiation, of ADSCs after adipocytic induction demonstrated that adipocytic differentiation of *Translin* KO ADSCs was enhanced.

N = 5 (B), 3 (C, D), or 4 (E).

Fig. 4. *In vitro* fertilized *Translin*$^{-/-}$ mice grow normally.

A. The weights of *in vitro*-fertilized *Translin*$^{-/-}$ and *Translim*$^{+/-}$ mice, derived from *Translin*$^{-/-}$ sperms and *Translim*$^{+/-}$ ova and fostered by pseudo-pregnant ICR mice, are shown. *In vitro*-fertilized *Translin*$^{-/-}$ mice did not show dwarfism as was reported for those of natural birth [13].

B and C. Basal metabolism of *in vitro*-fertilized *Translin*$^{-/-}$ mice, as assessed by body

temperatures (B) and food consumptions (C), showed no differences compared with that of *Translin*$^{+/-}$ mice..

D. Proposed role for Translin in mesenchymal cell physiology. Translin inhibits growth and differentiation of immature mesenchymal cells. It also possibly protects mesenchymal cells from epigenetic alterations caused by harsh environmental conditions.

N = 4-9 (A-C).

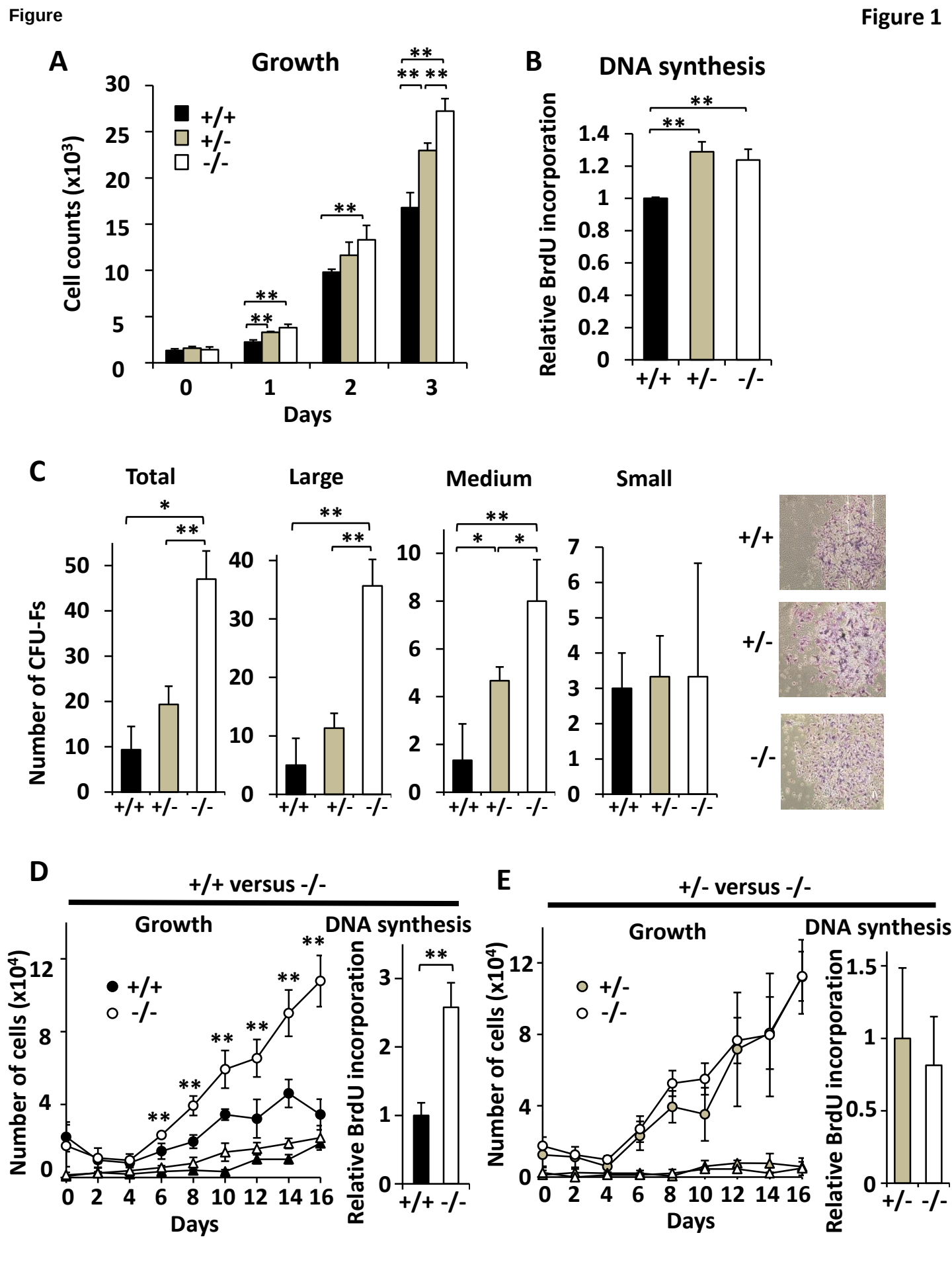
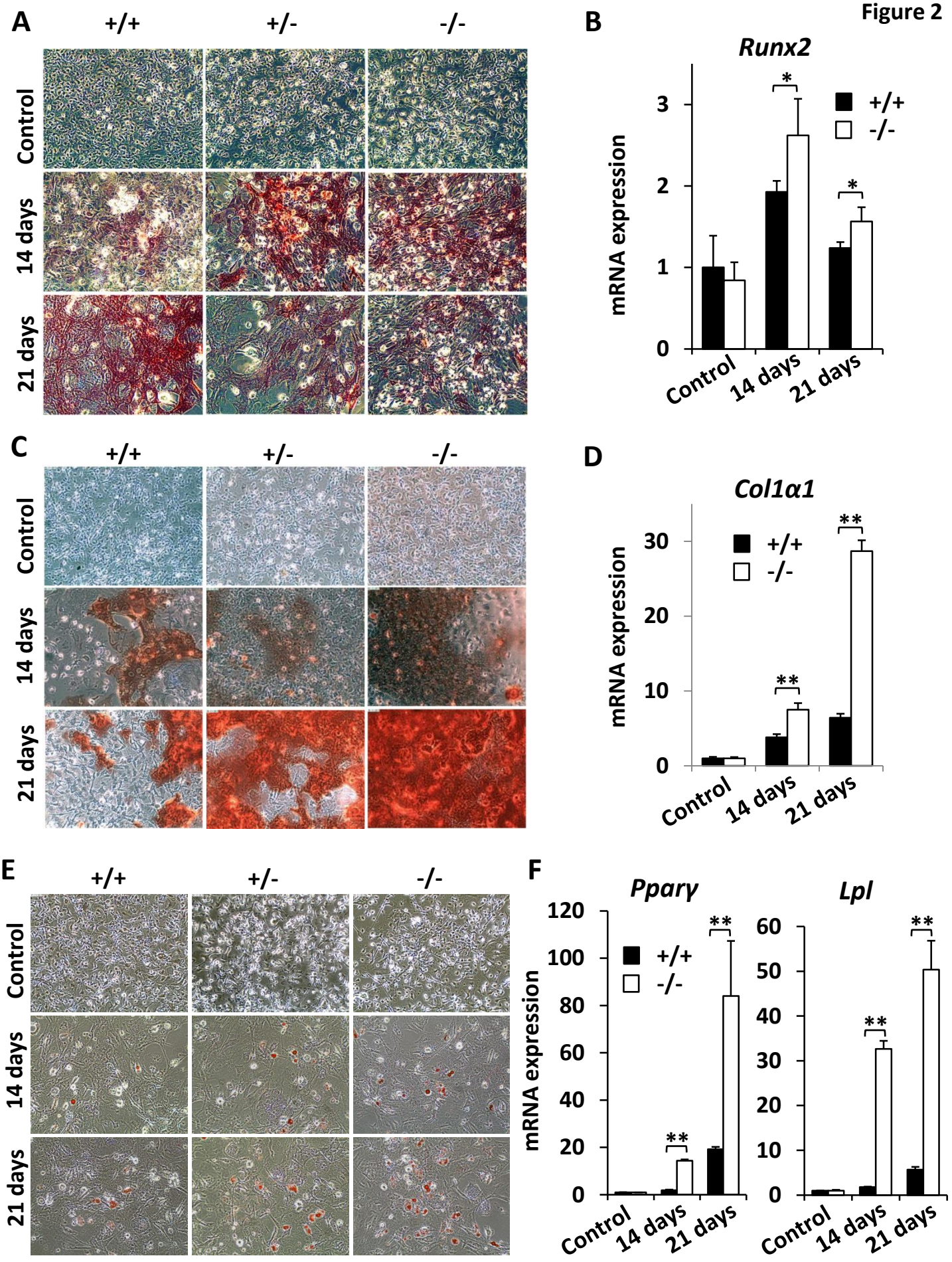
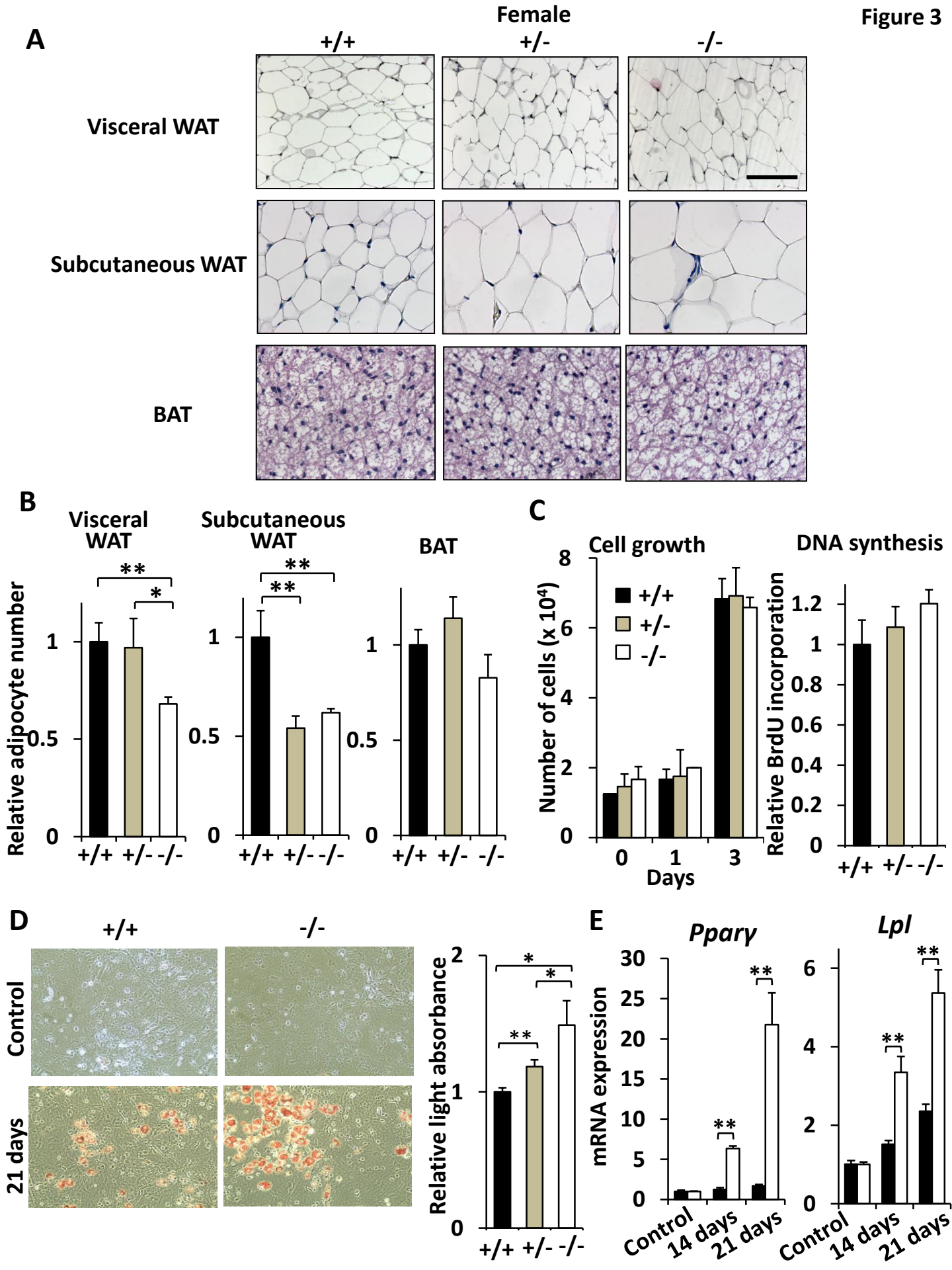
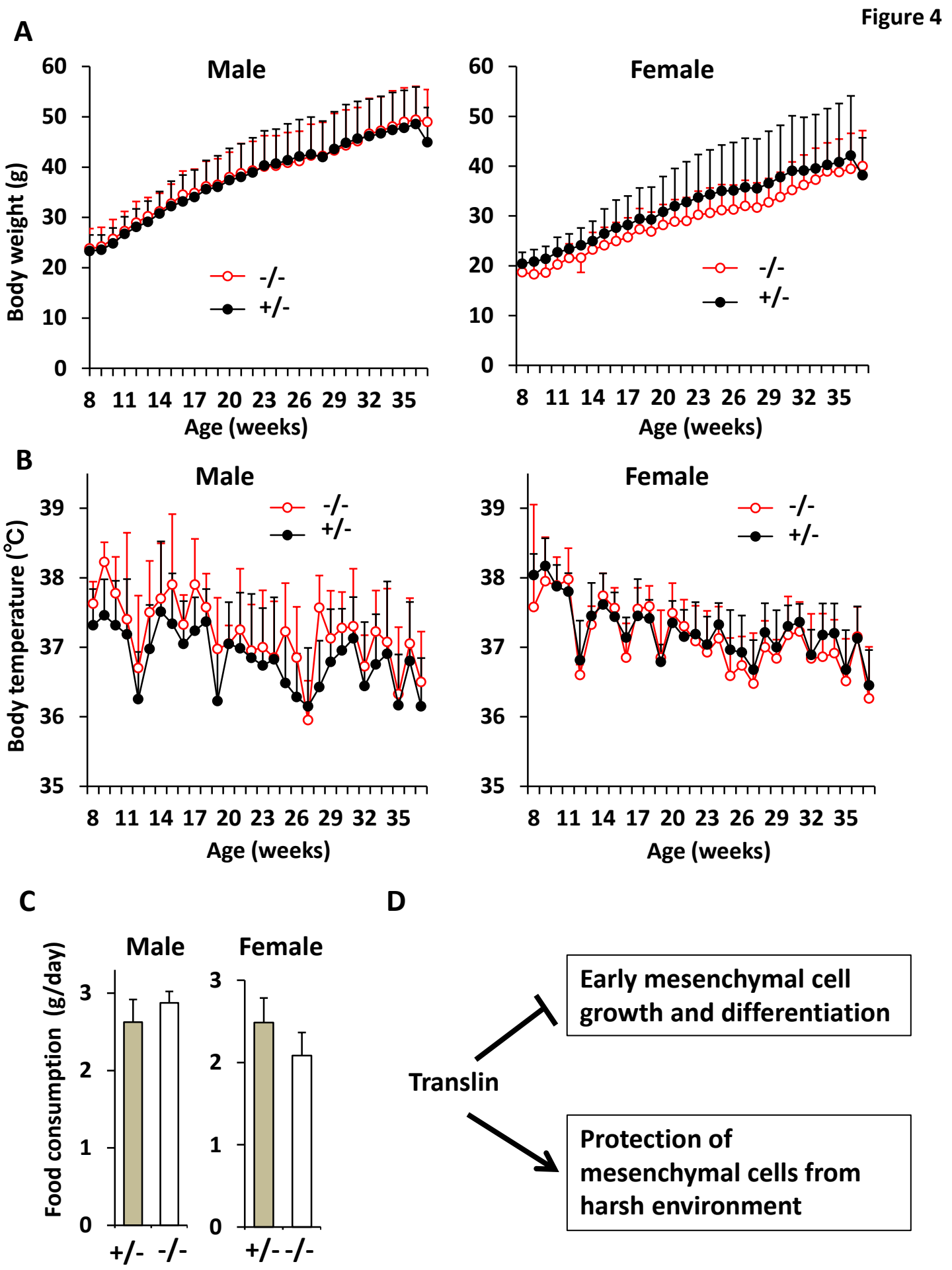
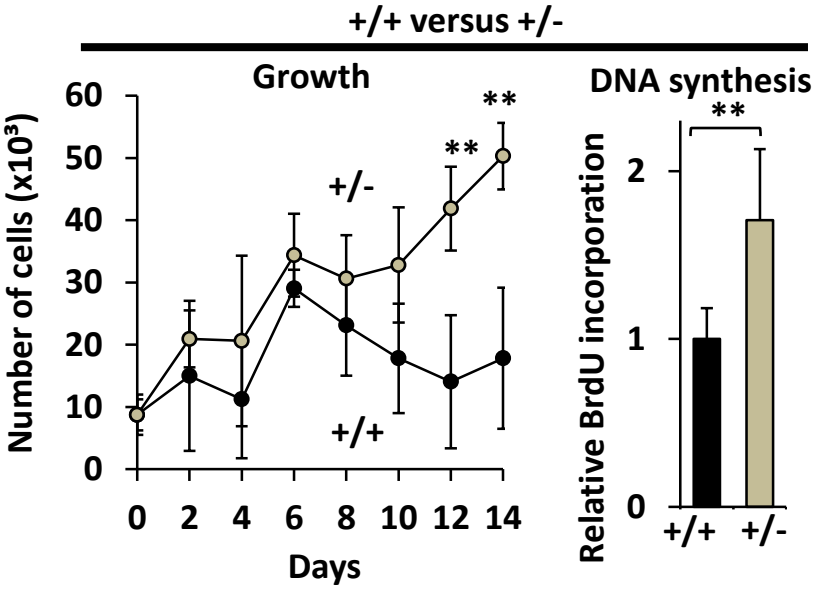

A
Growth
Cell counts (x10³)
+/+
+/-
-/-
Days
B
DNA synthesis
Relative BrdU incorporation
C
Total
Large
Medium
Small
Number of CFU-Fs
D
+/+ versus -/-
Growth
DNA synthesis
Number of cells (x10⁴)
Days
E
+/- versus -/-

Figure 2

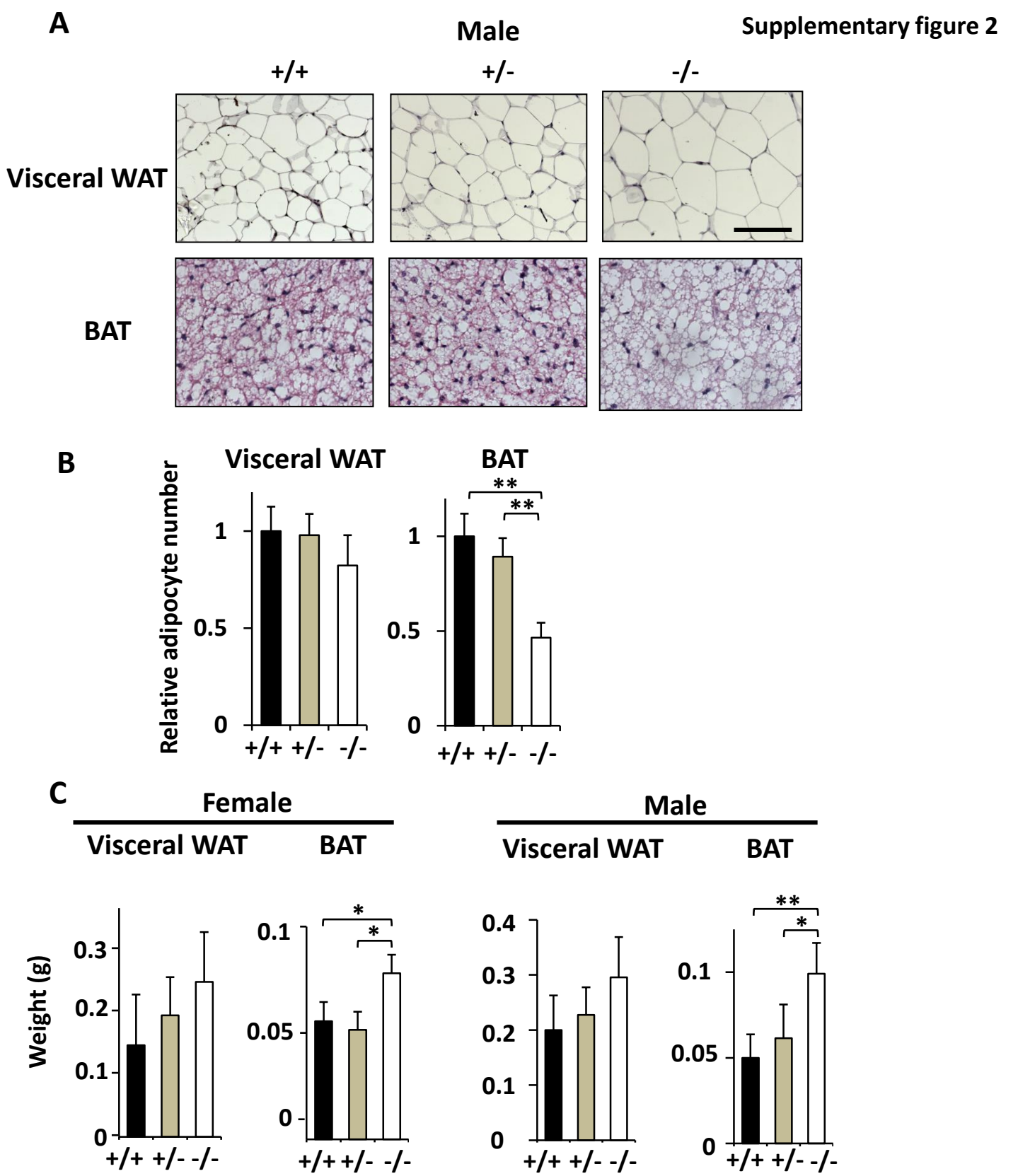

A
+/+
+/-
-/-
Control
14 days
21 days
B
Runx2
mRNA expression
+/+
-/-
Control
14 days
21 days
C
D
Col1α1
E
F
Pparγ
Lpl


A
Female
+/+
+/-
-/-
Visceral WAT
Subcutaneous WAT
BAT
B
Visceral WAT
Subcutaneous WAT
BAT
Relative adipocyte number
C
Cell growth
DNA synthesis
Number of cells (x 10⁴)
Relative BrdU incorporation
Days
D
Control
21 days
Relative light absorbance
E
Pparγ
Lpl
mRNA expression
Control
14 days
21 days


A
Male
Female
Body weight (g)
Age (weeks)
-/-
+/-
B
Body temperature (℃)
C
Food consumption (g/day)
+/-
-/-
D
Translin
Early mesenchymal cell growth and differentiation
Protection of mesenchymal cells from harsh environment



Supplementary Fig. 1. Growth (left panel) and DNA synthesis (right panel) of *Translin*$^{+/-}$ bome marrow (BM)-derived mesenchymal stem cells (MSCs) were enhanced compared with those of *Translin*$^{+/+}$ BM-MSCs, indicating that growth restricting function of Translin in BM-MSCs is haploinsufficient.
Average ± SD are shown. N = 3.



Supplementary Fig. 2. Translin restricts adipocytic differentiation and hypertrophy of adipose tissues.
A and B. Adipose tissues of 12-week-old male *Translin* KO mice. The numbers of adipocytes per unit area showed that *Translin* KO brown adipocytes were more hypertrophied than wild-type controls (B).
Bar, 100 μm.
C. *Translin*$^{-/-}$ brown adipose tissues (BATs) were heavier than the controls.
Average ± SD are shown (B, C). N = 10 (B) or 3-5 (C).