



# Cellular Proliferation of Equine Bone Marrow- and Adipose Tissue-Derived Mesenchymal Stem Cells Decline With Increasing Donor Age

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**Background:** Bone marrow (BM)- and adipose tissue (AT)-derived mesenchymal stem cells (MSCs) are used increasingly for autologous cell therapy in equine practice to treat musculoskeletal and other injuries. Current recommendations often call for 10–100 million MSCs per treatment, necessitating the expansion of primary cells in culture prior to therapeutic use. Of concern, human and rodent studies have shown a decline of both MSC recovery from sampled tissue and *in vitro* proliferative capacity with increasing donor age. This may be problematic for applications of autologous cell-based therapies in the important equine demographic of older patients.

#### **OPEN ACCESS**

#### Edited by:

Lauren Virginia Schnabel, North Carolina State University, United States

#### Reviewed by:

Alix Kay Berglund, North Carolina State University, United States Jennifer Michelle Cassano, Veterinary Medical Teaching Hospital, United States

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#### Specialty section:

This article was submitted to Veterinary Regenerative Medicine, a section of the journal Frontiers in Veterinary Science

Received: 03 September 2020 Accepted: 19 October 2020 Published: 10 December 2020

#### Citation:

Bagge J, MacLeod JN and Berg LC (2020) Cellular Proliferation of Equine Bone Marrow- and Adipose Tissue-Derived Mesenchymal Stem Cells Decline With Increasing Donor Age. Front. Vet. Sci. 7:602403. doi: 10.3389/fvets.2020.602403 **Objectives:** To investigate the effect of donor age on the cellular proliferation of equine BM- and AT-MSCs.

Study Design: In vitro study.

**Methods:** BM- and AT-MSCs and dermal fibroblasts (biological control) were harvested from horses in five different age groups (n = 4, N = 60); newborn (0 days), yearling (15–17 months), adult (5–8 years), middle-aged (12–18 years), and geriatric ( $\geq$ 22 years). Proliferation of the cells was tested using an EdU incorporation assay and steady state mRNA levels measured for targeted proliferation, aging, and senescence biomarkers.

**Results:** The cellular proliferation of equine BM- and AT-MSCs declined significantly in the geriatric cohort relative to the younger age groups. Proliferation levels in the two MSC types were equally affected by donor age. Analysis of steady state mRNA levels showed an up-regulation in tumor suppressors, apoptotic genes, and multiple growth factors in MSCs from old horses, and a down-regulation of some pro-cycling genes with a few differences between cell types.

**Main Limitations:** Potential age-dependent differences in cell function parameters relevant to cell-therapy application were not investigated.

**Conclusions:** The cellular proliferation of equine BM- and AT-MSCs declined at advanced donor ages. High levels of *in vitro* proliferation were observed in both MSC types from horses in the age groups below 18 years of age.

Keywords: horse, aging, donor age, mesenchymal stem cells, proliferation, tumor suppressors

## INTRODUCTION

Mesenchymal stem cells (MSCs) have shown potential to facilitate the repair of certain musculoskeletal and other tissue injuries, and are being used increasingly in equine practice (1–3). Cellular proliferation has been shown to be positively correlated with regenerative potential (4). Bone marrow (BM)- and adipose tissue (AT)-derived MSCs are currently the choice of therapy, where BM-MSCs have shown higher potential to treat cartilage and bone injuries (5, 6). For successful therapies, a substantial number of cells are needed, which often requires extensive *ex vivo* cell expansion prior to implantation. Generally, cell-based therapy protocols call for 10–100 million MSCs per treatment and are typically used for clinical applications at passage 3–4 (7–10). Additionally, repeated MSC applications have shown beneficial effects *in vivo*, which further increases the need for cells (11).

Unfortunately, human and rodent studies have shown both a decline in recovery from sampled tissues and a drop in the *in vitro* proliferative capacity of BM- and AT-MSCs with increasing donor age (12–15). The frequency of BM-MSCs is lower compared to AT-MSCs at isolation (16, 17). MSCs from aged donors have also been determined to have increased expression of cell cycle arrest genes like p53 and p21 (12, 14, 18), and decreased expression of growth factors like vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) (13).

Very little is known about these age-dependent relationships in horses. No equine BM-MSC donor age-dependent studies have been reported with multiple age groups to provide thresholds relevant for clinical practice, or have investigated if different MSC types are equally affected by donor age (16, 18–20). Expansion of cell numbers invariably causes a treatment delay, which may be an issue for some applications where early treatment has been shown to be beneficial (21). The inability to even achieve cell numbers recommended for therapy would likely have a far greater impact. Together, this may limit the clinical potential of MSCs from aged horses.

Presently, autologous treatment is preferred over allogenic treatment due to the risk of immunological reactions associated with allogenic treatments in equine models (22, 23). Clinical issues that may benefit from cell-based therapies occur across the full range of equine ages, including orthopedic problems in older sport and recreational horses (24). Together, this emphasizes the significance of understanding the effect donor age has on the proliferative capacity of equine MSCs.

The current study was, therefore, designed to test the hypothesis that increasing donor age is a major variable impacting equine BM- and AT-MSC proliferation with decreasing capacities. The aim of this study was to compare cellular proliferation and the expression of genes known to regulate cell proliferation in BM- and AT-MSCs from horses in five different age groups, and to test if the two MSC types were equally affected by donor age.

# MATERIALS AND METHODS

## **Experimental Samples**

Three different cell types, BM-MSCs, AT-MSCs, and dermal fibroblasts (DF) (biological non-stem cell control), were harvested as detailed below immediately post-mortem from horses of mixed breeds across five age groups. Four donor horses were used for each cell type and age group. The age groups were newborn (0 days old), yearling (15-17-month old), adult (5-8year old), middle-aged (12–18-year old), and geriatric ( $\geq$ 22-year old). DFs were chosen as a biological control due to their morphology and to have a non-stem cell type for comparison to potential MSC age-related changes. All horses were euthanized due to reasons unrelated to the current study. No systemic illness was apparent in any of the subjects, with the exception of some old horses as noted (Table 1). The study was conducted according to the ethical guidelines of animal research at the University of Copenhagen and the University of Kentucky. A written informed consent was obtained from all privately-owned horses prior to sample collection. Sample size was determined by two-sample t-test power analysis in R (version 3.6.0, The R Foundation for Statistical Computing, Vienna, Austria) using MSC proliferation pilot data (not shown) from newborn and geriatric horses. The power was set to 0.80 and the minimum relevant difference in proliferation rate was set to 10% between study groups. Experiments were performed at two different universities. An inter-laboratory control of BM-MSCs from the same yearling cell line was tested for proliferation rate and steady state mRNA levels at both sites after shipping RNA on dry-ice from one laboratory to the other to perform gene expression analyses on the same machines. No indication of significant differences between laboratories was found (data not shown). Plasticware, culture medium, reagents, commercially available kits, and protocols were kept constant throughout the entire study for all samples.

# Bone Marrow Derived MSC Collection and Isolation

BM was collected immediately post-mortem from the sternum of 12 female and 8 male horses (**Table 1**).

For newborns, BM was collected as described by Vidal et al. (16) with a few modifications. Briefly, BM samples were obtained from the 4th-6th sternebrae by sterile curettage after splitting the sternum along the midline. The marrow trabecular bone was transported on ice to the laboratory in sterile Dulbecco's phosphate buffered saline (dPBS, Thermo Fisher Scientific, Waltham, MA, USA) with 2% (v/v) amphotericin B (Thermo Fisher Scientific, Waltham, MA, USA) and 2% (v/v) penicillin/streptomycin (P/S, Thermo Fisher Scientific, Waltham, MA, USA) (isolation solution) and processed within 2h of collection. Marrow trabecular bone was rinsed twice in 37°C isolation solution and crushed before being grown as explant culture in two T75 flasks (Cellstar BioGreiner tissue culture treated flasks, Sigma-Aldrich, St. Louis, MI, USA) with 12 mL/flask Dulbecco's modified Eagle's medium (Gibco DMEM, 1 g/L glucose, with phenol red, GlutaMAX, and pyruvate,

Age group	Age	Breed	Gender	BM-MSCs	AT-MSCs	DFs	Reason for euthanasia
Newborn	0 d.	Pony	М	Yes	Yes	Yes	Research
	0 d.	Pony	Μ	Yes	Yes	Yes	Research
	0 d.	Pony	М	Yes	Yes	Yes	Research
	0 d.	Pony	F	Yes	Yes	Yes	Research
Yearling	15 mo.	Mixed, light breed	Μ	Yes	No	Yes	Research
	15 mo.	Mixed, light breed	М	No	Yes	Yes	Research
	15.5 mo.	Mixed, light breed	М	Yes	No	No	Research
	16 mo.	Mixed, light breed	F	Yes	Yes	Yes	Research
	16 mo.	Mixed, light breed	F	Yes	Yes	Yes	Research
	16 mo.	Mixed, light breed	М	No	Yes	No	Research
Adult	5 yo.	Standardbred	F	Yes	Yes	No	Research
	5 yo.	Standardbred	F	No	No	Yes	Research
	6 уо.	Standardbred	F	No	No	Yes	Research
	6 уо.	Standardbred	F	No	No	Yes	Research
	7 уо.	Standardbred	F	Yes	Yes	No	Research
	7 уо.	Standardbred	М	Yes	Yes	Yes	Research
	8 yo.	Warmblood	М	Yes	Yes	No	Unknown
Middle-aged	12 yo.	Standardbred	F	No	No	Yes	Research
	12 yo.	Standardbred	F	No	No	Yes	Research
	13 yo.	Standardbred	F	Yes	Yes	No	Research
	14 yo.	Pony	М	No	Yes	No	Unknown
	15 yo.	Standardbred	F	No	Yes	No	Research
	15 yo.	Standardbred	F	Yes	No	No	Research
	16 yo.	Pony	М	No	Yes	No	Unknown
	16 yo.	Thoroughbred	F	Yes	No	Yes	Research
	18 yo.	Thoroughbred	F	Yes	No	Yes	Weight loss
Geriatric	22 yo.	Thoroughbred	F	Yes	Yes	Yes	Research
	25 yo.	Coldblood	М	Yes	Yes	No	Unknown
	25 yo.	Thoroughbred	F	Yes	Yes	Yes	Lymphoma
	31 yo.	Thoroughbred	F	Yes	Yes	Yes	Colic
	32 yo.	Thoroughbred	F	No	No	Yes	Research

\*Research horses were euthanized for reasons unrelated to the current study and all were in apparent good health at the time of sacrifice. d., days old; mo., months old; yo., years old; M, male; F, female; BM, bone marrow; AT, adipose tissue; MSCs, mesenchymal stem cells; DF, dermal fibroblasts.

Thermo Fisher Scientific, Waltham, MA, USA), 10% (v/v) fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA), 1% (v/v) P/S, and 1% (v/v) amphotericin B (isolation medium). After 48 h, BM crusts and non-adherent cells were aspirated along with the isolation medium, and the medium was changed to expansion medium containing DMEM, 10% FBS, and 1% P/S.

For the four older age groups, 20 mL of BM aspirate was collected from the 4th-6th sternebrae with a 11G Jamshidi<sup>®</sup> BM needle (Henry Schein Vet, Dublin, OH, USA) and BM-MSCs were isolated with Ficoll-Paque<sup>®</sup> PREMIUM (GE Healthcare, Chicago, IL, USA) as previously described (16, 25).

The method used for sample collection of BM-MSCs varied between newborns and the other age groups because the smaller size and cartilaginous sternum of newborn foals proved challenging for the accurate positioning of Jamshidi<sup>®</sup> needles to obtain high quality bone marrow aspirates.

# Adipose Tissue Derived MSC Collection and Isolation

AT was collected and isolated from the gluteal region above the biceps femoris muscle next to the tail base of 10 female and 10 male horses (**Table 1**) as previously described (25).

Briefly, the gluteal area next to the tail base was surgically clipped and prepared, and 10 grams of AT was collected through a  $\sim$ 8 × 8 cm surgical window. AT was transferred to a 50 mL polypropylene tube (Falcon) with ice cold isolation solution and transported on ice to the laboratory, where the tissue was further processed within 2 h of collection. AT was washed two times in isolation solution and dissected into smaller pieces where visible blood vessels were removed. AT was digested in sterile filtered (0.2  $\mu$ m) enzyme medium consisting of DMEM (1 g/L glucose), 1% (v/v) P/S, 50  $\mu$ g/mL gentamycin (Sigma-Aldrich, St. Louis, MI, USA), and 1 mg/mL collagenase type I (Thermo Fisher Scientific, Waltham, MA, USA) for 3 h at 37°C and 30

rpm. Released cells were filtered through a 70  $\mu$ m cell strainer, washed twice in sterile dPBS, and centrifuged at 500 g for 5 min between the washes. The pellet was then re-suspended in 24 mL isolation medium supplemented with 50  $\mu$ g/mL gentamycin and distributed into two T75 flasks. Medium change to expansion medium occurred 48 h after isolation.

## **Dermal Fibroblast Collection and Isolation**

Unfortunately, DFs had not been collected originally from all donor horses for which archived primary BM and AT cell lines were available. To include this biological control cell type in the experimental design of the current study, additional donors were recruited in the appropriate age group as needed. DFs were harvested and isolated from the gluteal region above the biceps femoris muscle next to the tail base of 14 female and 6 male horses (**Table 1**) as previously described (6).

In short, approximately 6 grams of dermal tissue was collected from either the same surgical window generated to collect AT-MSCs or one comparably positioned. Dermis was transferred to a 50 mL polypropylene tube (Falcon) with ice cold isolation solution and transported on ice to the laboratory where the tissue was further processed within 2 h of collection. The dermal tissue was washed two times in isolation solution, dissected into smaller pieces, and digested in sterile filtered  $(0.2 \,\mu\text{m})$  enzyme medium consisting of dPBS, 1% (v/v) P/S, 1% (w/v) bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MI, USA), 50 µg/mL gentamycin, and 1 mg/mL collagenase type I for 2 h at 37°C and 30 rpm. Released cells were filtered through a 70  $\mu$ m cell strainer, washed twice in sterile dPBS, and centrifuged at 1,000 g for 4 min between the washes. The pellet was then re-suspended in 24 mL isolation medium supplemented with 50 µg/mL gentamycin and distributed into two T75 flasks and grown at 37°C in air with 5% CO<sub>2</sub>. A culture medium change to expansion medium was performed 48 h after isolation.

## **Cell Expansion and Storage**

The cells were cultured in expansion medium at  $37^{\circ}$ C in a humidified atmosphere containing 5% CO<sub>2</sub>. Expansion medium was changed every 2–3 days. At approximately 80% confluence, the cells were passaged with Trypsin/EDTA (Thermo Fisher Scientific, Waltham, MA, USA). Cell counting was performed manually using trypan blue (Thermo Fisher Scientific, Waltham, MA, USA) and a hemocytometer. The cells were grown with a seeding density of 500,000 cells per T75 flask. At passage 2, the cells were cryopreserved at a concentration of 2–3 million cells/mL freezing medium (Recovery-Cell Culture Freezing Medium<sup>®</sup>, Thermo Fisher Scientific, Waltham, MA, USA) in cryogenic vials (Nalgene, Thermo Fisher Scientific, Waltham, MA, USA).

For subsequent experimental applications, the cells were thawed in  $37^{\circ}$ C expansion medium and washed three times in dPBS before being plated and grown in expansion medium. As before, the cells were incubated at  $37^{\circ}$ C and 5% CO<sub>2</sub> with a medium change every 2–3 days. Proliferation and gene expression analyses were conducted with passage 4 cells.

## Assessment of Cellular Proliferation

Cellular proliferation was quantified by determining levels of incorporated 5-ethynyl-2'-deoxyuridine (EdU) after pulse labeling using a Click-iT Plus Alexa Fluor 594 EdU Imaging Kit<sup>®</sup> (Thermo Fisher Scientific, Waltham, MA, USA) as described previously (19).

In short, passage 4 cells were seeded at a density of 25,000 cells per well in a 24-well plate (Thermo Fisher Scientific, Waltham, MA, USA) and cultured in expansion medium for 48 h. The cells were then pulsed for 24 h with 8 µM EdU (Jena Bioscience, Jena, Germany). A total of eight technical replicate wells were pulsed with EdU, and one control well was kept under normal expansion medium without pulsing. Next, the cells were fixed in 4% methanol-free paraformaldehyde (Thermo Fisher Scientific, Waltham, MA, USA) and washed with 3% (w/v) BSA (Sigma-Aldrich, St. Louis, MI, USA) before being permeabilized with 0.1% Triton-X (Sigma-Aldrich, St. Louis, MI, USA). For EdU label detection, the cells were incubated for 30 min with the kit reagent cocktail containing Alexa 594. The staining cocktail was removed and the cells were washed with 3% BSA and dPBS before being counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Thermo Fisher Scientific, Waltham, MA, USA) for 15 min at a concentration of  $1 \mu g/mL$ . The fluorophore staining cocktail was prepared fresh for each assay and the cells were incubated while protected from light. Images of the cells were taken in the dark using a fluorescence microscope with DAPI and Alexa 594 filters. A total of three random images were taken per well for each fluorophore. The total number of cell nuclei and the number of proliferating cells were counted using automated imaging software (Image-J version 1.48, NIH, Bethesda, MD, USA). The cellular proliferation rate was calculated as the number of EdU labeled nuclei as a percentage of total cell nuclei in each image. The proliferation percentage was calculated for all three images per well and then averaged.

### **Differential Gene Expression** RNA Isolation and Reverse Transcription

Passage 4 cells were expanded in T75 flasks with expansion medium. At approximately 80% confluence, the cells were extracted with QIAzol® (Qiagen, Germantown, MD, USA) and snap frozen in liquid nitrogen before being stored at -80°C prior to RNA isolation. The cells were homogenized with a PowerGen 125 homogenizer (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was isolated using a Qiagen RNeasy Mini Kit<sup>®</sup> (Qiagen, Germantown, MD, USA) with modifications as previously described (26). RNA quantity was estimated with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) prior to ethanol precipitation. Purified RNA was quantified using a Qubit BR Assay (Thermo Fisher Scientific, Waltham, MA, USA) and NanoDrop spectrophotometer. The quality of the purified RNA was assessed with a Bioanalyzer 2100 (Eukaryotic Total RNA Nano & Pico Series II, Agilent Technologies, Santa Clara, CA, USA). All purified RNA samples met the following quality thresholds; 260/280 ratios of 1.9-2.1, 260/230 ratios of 1.8-2.28, and an Agilent RNA integrity number (RIN) of  $\geq$ 8.0, with the exception of one sample (RIN = 7.5) that behaved as expected in down-stream analyses and thus was not excluded from the study. Removal of potential genomic DNA contamination and reverse transcription of total RNA to cDNA was achieved using a commercially available kit as per manufacturer's protocol (Maxima First Strand cDNA Synthesis Kit<sup>®</sup> for RT-qPCR with dsDNase, Thermo Fisher Scientific, Waltham, MA, USA). All cDNA samples were diluted with nuclease-free water to 13.9 ng/uL and stored at  $-80^{\circ}$ C.

#### **Real-Time Quantitative PCR**

Forty seven biomarkers were selected for gene expression analyses based on functional annotation. Selected gene loci were chosen due to important biological relevance for cell proliferation, cellular senescence, or evidence of age-dependent variation related to proliferation in the literature. Commercially available, validated equine-specific TaqMan<sup>®</sup> primer-probe sets (Thermo Fisher Scientific, Waltham, MA, USA) (Table 2) for all biomarkers were used to quantitate steady state mRNA levels. The functionality of all primer-probe sets was tested against a positive control equine sample containing mixed cDNA from equal amounts of a 43-sample pool of various tissues (27), day 35 whole fetus, and neonatal epiphyseal cartilage. Negative controls of RNase-free water and minus-template were incorporated, and each sample was run in duplicate. Realtime quantitative PCR (RT-qPCR) reactions were conducted in a 384-well plate with 62.55 ng cDNA per reaction using a robotic ViiA<sup>TM</sup> RT-qPCR System (Thermo Fisher Scientific, Waltham, MA, USA). LinRegPCR was used to measure reaction amplification efficiencies and cycle threshold (Ct) values were calculated (28). All targets yielded amplification efficiencies close to 2 except for the negative controls that showed no amplification as expected. Three commercially available equinespecific endogenous control TaqMan<sup>®</sup> primer-probe sets; β-2microglobulin (B2M), β-glucoronidase (GUSB), and ribosomal protein lateral stalk subunit P0 (RPLP0), were tested against all samples. Using NormFinder software (Aarhus University Hospital, Aarhus, Denmark) (29), GUSB was determined as having the most uniform performance across all cell types and age groups.

Steady state mRNA levels from the selected gene loci were determined by RT-qPCR using the BIOMARK HD System (Fluidigm Corporations, South San Francisco, CA, USA) as previously described (30) at a cDNA concentration of 13.9 ng/ $\mu$ L. Negative controls and seven dilutions of the positive control sample described above were incorporated, each dilution being 3fold and ranging from 0.17 to 125 ng/µL. The Fluidigm protocol was carried out using the 96.96 Dynamic Array (Fluidigm Corporations, South San Francisco, CA, USA) according to manufacturer's instructions. Data were analyzed with Fluidigm Real-Time PCR Analysis Software in the BIOMARK instrument (Fluidigm Corporations, South San Francisco, CA, USA), where Ct values were calculated. Delta Ct values were determined for each sample by subtracting the corresponding Ct value of the endogenous control (GUSB). The positive control was used as a calibrator to calculate  $\Delta\Delta$ Ct values. Relative expression (RQ) of the gene targets were calculated using the  $2^{-\Delta\Delta Ct}$  method (31). RQ levels were used for graphical bar/boxplot presentations made in GraphPad Prism (version 8.0.1, GraphPad Prism, San Diego, CA, USA), and Ln(RQ) values were used for heatmap and statistical analyses (**Supplementary Table 1**).

For visualization of relative transcript levels between experimental groups, a heatmap was generated from the averaged Ln(RQ) levels, grouping the samples according to cell type and donor age, and genes according to biological function. The heatmap was prepared in R (version 3.6.0, The R Foundation for Statistical Computing, Vienna, Austria) using the *heatmap* ggplot function.

## **Statistical Analysis**

The cellular proliferation data, comparing age groups within cell types and across cell types, were analyzed with a generalized linear mixed model using The GLIMMIX Procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) with Tukey-Kramer's post hoc modifications for multiple comparisons. Gene expression data were analyzed in two steps. Initially, one-way analysis of variance (ANOVA) using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) was applied individually to all 47 gene targets within each cell type to look for donor age effects. Next, transcripts demonstrating significant differences by ANOVA and more than 5-fold difference between study groups on the heatmap were analyzed using The GLIMMIX Procedure with Tukey-Kramer's post hoc modifications for multiple comparisons to compare across tissue types. Normality of data was confirmed by QQ-plots and Shapiro-Wilk test. Statistical analyses of Fluidigm RT-qPCR results were performed on individual extracted Ln(RQ) values. Genes with missing data points (due to lack of detectable expression) were removed from the statistical analysis for the given cell type. To control for nonpaired samples and potential inter-laboratory variables, horse number and laboratory were added to the statistical models as additional factors. Data were considered statistically significant at p < 0.05.

## RESULTS

## **EdU Proliferation Assay**

All cell lines were able to be expanded to ~80% confluence at passage 4. When assessing the effect of donor age within one cell type, there was a significant decrease in cellular proliferation with increasing donor age for BM-MSCs (p = 0.02), AT-MSCs (p = 0.0003), and DFs (p < 0.0001) (**Figure 1**). Interestingly, there was no significant difference in pairwise comparisons between age groups other than geriatric horses for BM- and AT-MSCs (**Figure 1**). Representative images of proliferating BM-MSCs from horses in different age groups are shown in **Figure 2**.

All cell types were equally affected by donor age (p = 0.3) and no significant differences were observed in cellular proliferation between BM- and AT-MSCs in any age group (p > 0.4) (**Figure 3**). When comparing cell types independent of age groups, DFs had a higher overall level of cellular proliferation relative to BM-MSCs (p = 0.006) and AT-MSCs (p = 0.02). No Alexa 594 background staining was detected in any of the negative control wells grown without EdU pulsing. No study

#### TABLE 2 | Overview of TaqMan primer-probe sets used in RT-qPCR reactions.

ABSBP     ABS family member 3 binding protein     Decrease proliferation     Ex00002500,m1       AZP1     Arpaporin     Increase proliferation     Ex00002500,m1       ARED     Ampleguin     Micgonin effect     Ex000002500,m1       BARD     BPCA reasociated Namporal regulator     Pre-apportols     Ex00000000,m1       BAX     BC2 associated Xapporal regulator     Pre-apportols     Ex0000000,m1       BAX     BC3 associated Xapporal regulator     Pre-apportols     Ex0000000,m1       BAX     BC3 associated Xapporal regulator     Pre-apportols     Ex0000000,m1       BAX     Breake ancer type 1     Turnor suppressor     Ex0000000,m1       BAX     Catepase 3     Appototic gene     Ex0000000,m1       CARPS     Catepase 1     Pre-asse profileration     Ex0000000,m1       CARPS     Catepase issociated protein 1     Turno suppressor     Ex0000000,m1       CARPS	Gene ID	Gene name(s)	Gene function*	ThermoFisher Assay ID
AOPIAquaporinIncrease prelimentionEco8026426, mitAFEGAprilogianMitograic effectEco7001161, mitBAPO1BFOA1 associated X Apoptose regulatorPro-spoptolicEco7001161, mitBARABolic associated X Apoptose regulatorPro-spoptolicEco700000, gitBIRABolic associated X Apoptose regulatorPro-spoptolicEco700000, gitBIRABene morphogenic factor 3Increase preliferationEco700000, gitBIRABone morphogenic factor 3Apoptoto grainEco700000, gitCASPBCapase 3Apoptoto grainEco700000, gitCASPBCapase 3Apoptoto grainEco700000, gitCASPBCapase 3Apoptoto grainEco700000, gitCANNACapase 3Apoptoto grainEco7000000, gitCANNACapase apolitanciaEco7000000, gitEco7000000, gitCANNACapase apolitanciaEco7000000, gitEco7000000, gitCANNACapase apolitanciaEco7000000, gitEco7000000, gitCANNACapase apolitanciaEco7000000, gitEco7000000, gitCANNAEco7000000, gitEco7000000, gitEco7000000, gitCANNAEpitebel mitigs protin factor 1MotoganEco7000000, gitCANNA	ABI3BP	ABI family member 3 binding protein	Decrease proliferation	Ec06625599_m1
AFEGAmplinguinMtogenic effectEckB002086,m1BAPO1BRO1BCD2 associated X4 poptioss regulatorPro-apopticEc07016716_s1BAXBCL2 associated X4 poptioss regulatorPro-apopticEc0702080.g1BDR3Born morphogenic factor 3Immor suppressorEc0702080.g1BIRABread cancer type 1Tumor suppressorEc0701788.g1CASPSCapase 3Apoptiol goneEc0703768.m1CASPSCapase 3Apoptiol goneEc0703807.g1CASPSCapase 3Apoptiol goneEc080581.g1CANN1Quente associated protein 1Indice senescanceEc080581.g1CANN1Caudias associated protein 1Tumor suppressorEc080581.g1CANN1DataPro-sycing geneEc080581.g1CANN1DataPro-sycing geneEc080581.g1CANN1DataPro-sycing geneEc080581.g1CANN1DataPro-sycing geneEc080581.g1CANN1DataPro-sycing geneEc08059.g1CANN1DataEc08057.g1Ec08057.g1CAGPCardiago digonetic factorMtogenEc08057.g1CTAFFibrobal growth factor 1MtogenEc08057.g1CTAFFibrobal growth factor 18MtogenEc08057.g1CTAFFibrobal growth factor 18MtogenEc08057.g1CTAFFibrobal growth factor 18MtogenEc08057.g1CTAFFibrobal growth factor 18MtogenEc08057.g1CTAFFibrobal growth factor 18	AQP1	Aquaporin	Increase proliferation	Ec06625425_m1
BANDBICA1-associater filmS domain 1Tumor suppressorFoU/001151_minBAXBCA1-associater X apoptoins regulatorPorspaptoinEx07058800_g1BCL2Biol hymphona 2Anti-aspotoinEx07058800_g1BCR3Bore morphogenie factor 3incease preliferationEx07058800_g1BCR4Bore morphogenie factor 3incease preliferationEx0707788_m1BCR4Bore anone type 1Incease preliferationEx087031_m1CASP8Capase 3Accolotic paneEx087031_m1CANN1Cavolote bassociated protoin 1Holos servacenceEx070587_m1CANN1Cavolote bassociated protoin 1Indro servacenceEx070587_m1CAN14Cavolote bassociated protoin 1Tumor suppressorEx080657_m1CAN14CaluatoriParticipage oligomatic matrix proteinIntro-suppressorEx080657_m1CAN14CaluatoriEx080657_m1Ex080657_m1Ex080657_m1CAN14CaluatoriEx080657_m1Ex080657_m1Ex080657_m1CATFColory stimulating fator 2Increase preliferationEx080657_m1CATFColory stimulating fator 3MitogenEx080697_m1CATFColory stimulating fator 1MitogenEx080697_m1CATFColory stimulating fator 1MitogenEx080697_m1CATFColory stimulating fator 1MitogenEx080697_m1CATFColory stimulation fator 1MitogenEx080697_m1CATFColory stimulation fator 1MitogenEx080697_m1CATF	AREG	Amphiregulin	Mitogenic effect	Ec06992855_m1
BAXBCL2 associated Xapptosis regulatorPro-apptopicEc0706180.BCL2Borne mapphagenic factor 3Increase proliferationEc0706180	BARD1	BRCA1-associated RING domain 1	Tumor suppressor	Ec07061151_m1
BCL2B-nell lymphome 2Anti-seprelationE-007087068_m1BMPGBone morphogenic factor 3increase proliferationE-007087068_m1BMPGCaspase 3Apoptolic geneE-007087068_m1CASPBCaspase 3Apoptolic geneE-007087068_m1CANNACaspase 3Apoptolic geneE-007087067_m1CANNACapocoa associated protein 1Indoor associanceE-00708807_m1COND1Caylin D1Indoor associanceE-00708807_m1COND1OutliefAnti-sepptolicE-0084806_m1CLUOutliefAnti-sepptolicE-0048807_m1CLUOutliefIncrease proliferationE-0048807_m1CSP2Calory stimulating factor 2Increase proliferationE-0048807_m1CGFConnective tassue growth factor 1Micagen 1E-0048807_m1CGFE-00488107_m1MicagenE-0048807_m1FGF1Broobast growth factor 1MicagenE-0048807_m1FGF1Broobast growth factor 1MicagenE-0048807_m1FGF1Broobast growth factor 1MicagenE-00582480_m1FGF3Broobast growth factor 1MicagenE-00682751_m1FGF4Broobast growth factor 1MicagenE-00682751_m1FGF4Broobast growth factor 1MicagenE-00703712_m1FGF4Broobast growth factor 1MicagenE-00703771_m1FGF4Broobast growth factor 1MicagenE-00703771_m1FGF4Broobast growth factor 1MicagenE-00703771_m1	BAX	BCL2 associated X apoptosis regulator	Pro-apoptotic	Ec07016716_s1
BNRS.Bore morphogenic factor 3Increase proferentionE-0070768E_n1BRGA1Breast cancer type 1Junor suppressorE-0070768E_n1CASP3Catapase 3Apostotic geneE-008081A1_m1CASP4Catapase 8Apostotic geneE-00708872.m1CASP4Catapase 8Apostotic geneE-00708872.m1CON14Quilo D1Holes servescenceE-00708872.m1CON14Quilo D1UterinHoles servescenceE-00708872.m1CON1Catafage dependent kinase inhibitor 1ATuror suppressorE-008985.18.m1CON2Catafage dependent kinase inhibitor 1AIncrease profilerationE-008985.01.m1CON2Catafage dependent kinase inhibitor 1AIncrease profilerationE-006982589.m1CTGFConnective tissue growth factor 2Increase profilerationE-00698277.gHCTGFConnective tissue growth factor 1MitogenE-006982788.m1FGF1Faroblast growth factor 1MitogenE-006982788.m1FGF3Faroblast growth factor 1MitogenE-006982788.m1FGF4Faroblast growth factor 1MitogenE-00700771.m1FGF5Faroblast growth factor 1MitogenE-00700771.m1FGF6Hanoblast growth fac	BCL2	B-cell lymphoma 2	Anti-apoptotic	Ec07005800_g1
BinCAPreast cancer type 1Turner suppressorEx000470891_m1CASPBCaspase 3Apoptotic geneEx00470091_m1CASPBCaspase 8Apoptotic geneEx00470091_m1CANN1Caveoiae associated protein 1Induce sensecuresEx0708867_m1COND1Cycle 01Pro-sycling geneEx0708867_m1CONTAQuitering generic matrix proteinIncrease profilerationEx0848857_m1COMPCautage algormaric matrix proteinIncrease profilerationEx0848867_m1COMPCautage algormaric matrix proteinIncrease profilerationEx0848867_m1CIFGFCatory stimuling factor 2Increase profilerationEx0848867_m1CTINE1Bat-caterinIncrease profilerationEx0848877_m1FEFEpitoelan factor 1MitogenEx0148877_m1FOFIBBrooblast growth factor 5MitogenEx0148877_m1FOFIBBat-caterin factor 6Turner suppressorEx07039714_m1GDF6Gowth differentiation factor 6Turner suppressorEx07039711_m1GDF6Handamed growth factorMitogenEx0703775_m1HGFHapatoma-derined growth factorMitogenEx0703775_m1HGFHapatoma-derined growth factorMitogenEx0703775_m1HGFHapatoma-derined growth factor 1MitogenEx0703775_m1HGFHapatoma-derined growth factor suburnMitogenEx0703777_m1HGFHapatoma-derined growth factor suburnMitogenEx0703777_m1HGFHapatoma-derined gr	BMP3	Bone morphogenic factor 3	Increase proliferation	Ec07037656_m1
CASP3Caspase 3Apoptolic geneEC0497/091_m1CASP6Caspase 8Apoptolic geneEc04956913_m1CAND1Cavelee associated protein 1Induce senescienceEc07050937_m1CCND1Cyclin D1Pro-cycling geneEc07050937_m1CLW1OlusterinIruner suppressorEc09956195_m11CLW1OlusterinIrunesse profilerationEc09956195_m11CLW1OlusterinIruner suppressorEc09956195_m11CLW2Cartilge oligometic matrix proteinIncrease profilerationEc09961910_m1CTFFConnective staue growth factorIncrease profilerationEc09961910_m1CTFFConnective staue growth factorMtogenEc09961910_m1FGF1Fibroblast growth factor 18MtogenEc01906277_m1FGF5Fibroblast growth factor 18MtogenEc0496777_m1FGF6Fibroblast growth factor 18MtogenEc0496777_m1FGF1Bitroblast growth factor 18Benescence markerEc0596183_m1GLB1Bitroblast growth factor 18Benescence markerEc059619_m1GLF6Fibroblast growth factorMtogenEc07000731_m1HGFHepatom-derved growth factorMtogenEc07070711_m1HGFInstituter fibribing protein 5MtogenEc07070711_m1IGFFInstituter fibribing protein 5MtogenEc07070711_m1IGFFInstituter fibribing protein 5MtogenEc07070771_m1IGFFInstituter fibribing protein 5MtogenEc07070771_m1 </td <td>BRCA1</td> <td>Breast cancer type 1</td> <td>Tumor suppressor</td> <td>Ec07017862_s1</td>	BRCA1	Breast cancer type 1	Tumor suppressor	Ec07017862_s1
CASPBCaspase BApplotic greeEco300809413_m1CAMN1Caveolae associated protein 1indce senesconceEc07008073_m1CAND1Cyclip D1Pro-cycling greeEc03708093_m1CDKN1A21, Cyclin dependent kinase inhibitor 1AUmor suppressorEc03488075_m1CLUCartlage digometic matrix proteinIncrease proteinationEc03488075_m1CDKPCartlage digometic matrix proteinIncrease proteinationEc03488075_m1CSF2Colony stimulating factor 2Increase proteinationEc03488075_m1CSF2Colony stimulating factor 2Increase proteinationEc0368077_p1CTNNB1Beta-cateninIncrease proteinationEc0368077_p1CTNNB1Beta-cateninMitogenEc03080773_m1FGF1Eroblast growth factor 1MitogenEc03080773_m1FGF3Eroblast growth factor 18MitogenEc03080774_m1GDF6Elonablast growth factor 18MitogenEc03080771_m1GLB1Bdt-galaccioladaseSenesconce markerEc09087112_m1GLF6Hepators/e growth factorMitogenEc0300054_m1GLF7Hepators/e growth factorMitogenEc03700751_m1HGFHepators/e growth factorMitogen bindingEc0370771_m1HGFHepators/e growth factor Sinding protein 5Mitogen bindingEc0370771_m1HGFHepators/e growth factor Sinding protein 5Mitogen bindingEc0370771_m1HGFHepators/e growth factor Sinding protein 5Mitogen bindingEc03970771_m1<	CASP3	Caspase 3	Apoptotic gene	Ec03470391_m1
CAVM11Caveolae secolated protein 1Indice sensescenceE07030873_m1COND1OptionPro-opting genomeE07030874COND1OptionPro-opting genomeE07030874CLUClusterinIumor suppressorE00346875CLUClusterinIncrease profilerationE00346875CSP2Colon strukting factor 2Increase profilerationE00346873CTGFConnective tissue growth factorIncrease profilerationE00396893CTGFConnective tissue growth factorIncrease profilerationE00396193EFGNEnthelia mitogenMitogenE00396193FGF5Fibroblast growth factor 1MitogenE00396193CGF6Fibroblast growth factor 16MitogenE00396193CGF6Fibroblast growth factor 16MitogenE00396177.1m1FGF1Bat-galactosiciaseSenescence markerE00396193CLB1Bat-galactosiciaseSenescence markerE00396193CLG1Hipethon-adversed growth factorMitogenE0039712.1m1GGF6Insult-Nike growth factor 1MitogenE0039713GGF8Insult-Nike growth factor 1MitogenE0039713.1m1GGF8Insult-Nike growth factor 1MitogenE0039713GGF8Insult-Nike growth factor 1MitogenE0039713.1m1GGF8Insult-Nike growth factor 1MitogenE0039713.1m1GGF8Insult-Nike growth factor 1MitogenE0039713.1m1GGF8Insult-Nike growth factor 1Mitog	CASP8	Caspase 8	Apoptotic gene	Ec06959413_m1
CXND1Qvdin D1Pro-cycling geneEx07036986_m1CDNN1Ap21, Cyclin-dependent kinase inhibitor 1ATumor suppressorEx06955195_m1CUUClatterinAnita-goptaloEx02468082_m1COMPCartlage cligomeric matrix proteinIncrease proliferationEx02468082_m1CSF2Colony stimulating factor 2Increase proliferationEx02468082_m1CTNRDenote:the issue growth factorIncrease proliferationEx026929_m1CTNRBeh-cateinIncrease proliferationEx026929_m1CTNREpithelal mitogenMitogenEx0246827_m1FGF1Bibrobiast growth factor 5MitogenEx02468774_m1FGF18Bibrobiast growth factor 6Tumor suppressorEx02468774_m1GDF6Growth differantiation factor 6Tumor suppressorEx0246877_m1GLF6Hepstanger digrowth factorRegulate proliferationEx0246877_m1GLF6Hepstanger digrowth factorMitogenEx0246803_m1GLF6Hepstanger digrowth factorMitogenEx0246874_m1HOGFHepstanger growth factorMitogenEx0246804_m1HOGFHepstanger growth factorMitogenEx0246869_m1LC10148270p16_bc_Qdin-dependent Kinase 4 inhibitor BSensecence markerEx0703741_m1HOGFHepstanger exores for tyrosine kinaseRegulate proliferationEx0247286_m1LC10148270p16_bc_Qdin-dependent kinase 4 inhibitor BSensecence markerEx0703741_m1HOGFHepstanger exores for tyrosine kinaseR	CAVIN1	Caveolae associated protein 1	Induce senescence	Ec07036873_m1
CDKN1Ap21, Cyclin-dependent kinase inhibitor 1ATuror suppressorEC08965185_m1CLUClusterinAnti-apoptolicEC0840807,m1CDMPCathligo oligonaric matrix proteinIncrease proliferationEC0840802,m1CSF2Colony stmulating factor 2Increase proliferationEC0892171_gHCTGFConnective tissue growth factorIncrease proliferationEC0892189_m1EPGNEpithalial matogenMtogenEC089289_m1EPGNEpithalial motogenMtogenEC089278_m1FGF3Fibroblast growth factor 18MtogenEC089269_m1GDF6Growth differentiation factor 6Umor suppressorEC089269_m1GLB1Beta-galactosideseSenescone markerEC0892618_m1GLB1Beta-galactosideseSenescone markerEC0892618_m1GLG2GLI famity zine finger 3MtogenEc0703715_m1HG6Hepatcoryle growth factorMtogenEc0703751_m1HG7Isalin-like growth factor 1MtogenEc07037751_m1IGFP5Isalin-like growth factor 1MtogenEc07037751_m1IGFP6Isalin-like growth factor binding protein 5MtogenEc07037751_m1IGFP5Isalin-like growth factor binding protein 5MtogenEc07037751_m1IGFP6Isalin-like growth factor binding protein 6Senescone markerEc082441_m1IGFP7Isalin-like growth factor binding protein finatorEc0892741_m1IGFP6Isalin-like growth factor binding protein finatorEc0892741_m1IGFP6 <td>CCND1</td> <td>Cyclin D1</td> <td>Pro-cycling gene</td> <td>Ec07036996_m1</td>	CCND1	Cyclin D1	Pro-cycling gene	Ec07036996_m1
CLUQueterinAnti-apoptoticEc0846875_m1COMPCarllage digomeric matrix probinIncrease proliferationEc0846808_m1CSF2Colony simulating factor 2Increase proliferationEc08260_m1CTGFConnective lissue growth factorIncrease proliferationEc082677_gH1CTNNB1Beta-cateninIncrease proliferationEc089269_m1FGFFabrelist mitogenMitogenEc0102738_m1FGF1Floroblast growth factor 5MitogenEc04086774_m1GDF6Growth fifterentistion factor 6Tumor suppressorEc07097112_m1GL1Beta-galactoxidaseSenescence markerEc0893883_m1GL3GL Itamily zinc firger 3Reguiter proliferationEc07097711_m1GDF6Hepatonyda growth factor 1MitogenEc0709771_m1IGF1Insulin-like growth factor 1MitogenEc0709771_m1IGF2Insulin-like growth factor 1Mitogen bindingEc0709771_m1IGF1Insulin-like growth factor 1Mitogen bindingEc0709771_m1IGF2Insulin-like growth factor 1Mitogen bindingEc0709731_m1IGF2Insulin-like growth factor 1Mitogen bindingEc0709731_m1IGF2	CDKN1A	p21, Cyclin-dependent kinase inhibitor 1A	Tumor suppressor	Ec06955195_m1
COMPCartilage oligomeric matrix proteinIncrease proiferationEc03468082_m1CSF2Colonsy disubating factor 2Increase proiferationEc03468082_m1CTGFConnective issues growth factorIncrease proiferationEc00991819_m1EFNABata-caterinIncrease proiferationEc00991819_m1EFNAEtholatin mtogenMtogenEc00991819_m1FGF1Fibroblast growth factor 15MtogenEc04656774_m1FGF3Fibroblast growth factor 5MtogenEc04656774_m1GDF6Growth differentiation factor 6Turo suppressorEc06954883_m1GLB1Bata-galactosidaseSenesconce markerEc06954883_m1GLB1GLI family zine frager 3MtogenEc07007512_m1HGFHepatona-derived growth factor 1MtogenEc07007512_m1HGFInsuin-like growth factor 1MtogenEc070037751_m1HGFBP5Insuin-like growth factor 1MtogenEc070037781_m1LGFBP5Insuin-like growth factor 1MtogenEc07003710_m1LGFBP5Insuin-like growth factor 1MtogenEc07037781_m1LGFBP5Insuin-like growth factor subunit DEc0897471_m1LGFBP5Insuin-like growth factor 1Ec0897471	CLU	Clusterin	Anti-apoptotic	Ec03468575_m1
CSF2Colon stimulating factor 2Increase proliferationEc03468208_m1CTGFConnective tissue growth factorIncrease proliferationEc0682577_gHCTNNE1Beta-cateninMitogenEc0692859_m1EPGNEpitoblast growth factor 1MitogenEc0492859_m1FGF1Floroblast growth factor 5MitogenEc04569774_m1FGF18Floroblast growth factor 13MitogenEc0492859_m1GDF6Growth differentiation factor 6Tumor suppressorEc0692512_m1GDF6Growth differentiation factor 6Tumor suppressorEc0692512_m1GDF6Gu family and finger 3Senescence markerEc0692512_m1GDF6Hapatocyte growth factor 1MitogenEc0703771_m1HGFHapatocyte growth factorMitogenEc0703771_m1HGFHapatocyte growth factor binding protein 5MitogenEc0703771_m1HGFInsulinike growth factor binding protein 5MitogenEc0703771_m1MTCo-mycPro-cyclingEc0703771_m1MTCo-mycPro-cyclingEc0703771_m1MTCo-mycPro-cyclingEc0703771_m1MTCo-mycPro-cyclingEc0703771_m1PCNAPoliferating cell nuclear antigenIncrease DNA replicationEc0692241_m1MTCo-mycPro-cyclingEc0703711_m1PCNAPoliferating cell nuclear antigenIncrease DNA replicationEc06927412_m1PGFDPoliferating cell nuclear antigenIncrease DNA replicationEc06922441_m1 </td <td>COMP</td> <td>Cartilage oligomeric matrix protein</td> <td>Increase proliferation</td> <td>Ec03468062_m1</td>	COMP	Cartilage oligomeric matrix protein	Increase proliferation	Ec03468062_m1
CTGFConnective tissue growth factorincrease proliferationEc06982577_gPICTNRE1Beta-ateninincrease proliferationEc0099181g_m1FGFNEpithelial milogenMitogenEc00192738_m1FGF1Fibroblast growth factor 1MitogenEc04685774_g11FGF5Fibroblast growth factor 5MitogenEc04685774_g11GDF6Growth differentiation factor 6Turor suppressorEc04692738_m11GLB1Beta-galactosidaseSenescence markerEc069248217_g11GLG5Hapatoma-derived growth factorMitogenEc07097712_m11HGFHepatoma-derived growth factorMitogenEc0703775_m11HGFHepatoma-derived growth factorMitogenEc0703775_m11IGF1Insulin-like growth factorMitogenEc03048683_m1IGFBP5Insulin-like growth factor 1Mitogen londingEc03073771_m1IGFBP5Insulin-like growth factor 1Mitogen londingEc03047026_m1IGFBP5Insulin-like growth factor 1Mitogen londingEc0703711_m1IGFBP5Insulin-like growth factor 1MitogenEc0703711_m1IGFBP5Insulin-like growth factor subunit DSenescence markerEc0703711_m1IGFBP5Insulin-like growth factor subunit DMitogenEc0703711_m1PCNAProfocioncogene, receptor tyrosine kinasePro-yolingEc0703711_m1PCNAProfobitinEc0703711_m1Ec0703711_m1PCNAProhabitinProfobitinEc0939714_m1PGFDProhabiti	CSF2	Colony stimulating factor 2	Increase proliferation	Ec03468208_m1
CTNNB1Beta-cateninIncrease proliferationEc00991819_m1EPGNEpithelial mitogenMitogenEc00992859_m1FGF1Fibroblast growth factor 1MitogenEc04868774_m1FGF5Fibroblast growth factor 18MitogenEc04868774_m1GDF6Growth differentiation factor 6Tumor suppressorEc03948217_g1GDF6Growth differentiation factor 6Tumor suppressorEc03948217_g1GL3GUI family zinc finger 3Regulate proliferationEc06964363_m1GL3GUI family zinc finger 3Regulate proliferationEc06926512_m1HDGFHepatocyte growth factor 1MitogenEc0700751_m1HGFInsulin-like growth factor 1MitogenEc03707751_m1IGF1Insulin-like growth factor 1MitogenEc03707751_m1IGF2Insulin-like growth factor 1MitogenEc03707751_m1METMET proto-onogene, receptor byosine kinaseRegulate proliferationEc0262241_m1MYCc-mycPro-cyclingEc0700751_m1PGFDPatelet-derived growth factor subunit DInforease DN explatissEc0709751_m1PGFDPatelet-derived growth factor subunit DInforease DN explatissEc0370751_m1PGFDPatelet-derived growth factor subunit DInforease ProliferationEc03468689_m1SOSTScheering onlinear antigenInforease ProliferationEc03469132_m1PGFDPatelet-derived growth factor subunit DInforeaseorEc0692622_m1SOSTScheering onlinear antigen	CTGF	Connective tissue growth factor	Increase proliferation	Ec06625777_gH
EPGNEptihelal mitogenMitogenEco699285_m1FGF1Floroblast growth factor 1MitogenEco1092738_m1FGF5Floroblast growth factor 5MitogenEco3948217_g1GDF6Growth differentiation factor 6Tumor suppressorEco3948217_g1GDF6Growth differentiation factor 6Tumor suppressorEco69625612_m1GLB1Beta-galactoxidaseSenescence markerEco69625612_m1HDGFHepatome-drived growth factorMitogenEco7037751_m1HGFHepatome-drived growth factorMitogenEco7037751_m1IGF1Insulin-like growth factor 1MitogenEco7037751_m1IGFEP5Insulin-like growth factor 1MitogenEco3468689_m1LOC100146270p16, Oxlin-dependent kinase 4 inhibitor BSenescence markerEco7037471_mHMTMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEco89262241_m1POSPDp16 berlinEco1402086_m1Eco692714_m1POSPDPiabel-derived growth factor subunit DMitogenEco692714_m1POSPDPiabel-derived growth factor subunit DMitogenEco69271_m1POSPDPiabel-derived growth factor subunit DMitogenEco69271_m1POSPDPiabel-derived growth factor bataRegulate proliferationEco6925392_m1SOSTSolo Calcum binding protein A1Inhibits DNA synthesisEco692537_m1SOSTSolo Calcum binding protein A1Nutror suppressorEco6925377_m1TGFATransforming growth factor bata 3 </td <td>CTNNB1</td> <td>Beta-catenin</td> <td>Increase proliferation</td> <td>Ec00991819 m1</td>	CTNNB1	Beta-catenin	Increase proliferation	Ec00991819 m1
FGF1Fibroblast growth factor 1MitogenEc0109273_m1FGF5Fibroblast growth factor 5MitogenEc04656774_m1FGF18Fibroblast growth factor 8MitogenEc03248217_g1GDF6Growth differentiation factor 6Tumor suppressorEc07097112_m1GLB1Beta-galactosidaseSenescence markerEc06954383_m1GLB2GL family zho finger 3Regulate proliferationEc0700751_m1HGFHepatoma-drived growth factorMitogenEc0700054_m1HGF1Insulin-like growth factor 1MitogenEc0700054_m1LGF10Insulin-like growth factor 1Mitogen bindingEc03476868_m1LGF10Insulin-like growth factor 1Mitogen bindingEc03470296_m1LGC100146270p16, Oyelin-dependent kinase 4 inhibitor BSenescence markerEc07007711_m1MFTMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc08262441_m1MYCc-mycPro-gropilerationEc07007711_m1PGFDPatched 2Tumor suppressorEc07007511_m1PGFDPatched 2Tumor suppressorEc06927714_m1PGF20Patched 2Tumor suppressorEc0682597_m1SOSTScherostinScherostinEc0692714_m1TGFATransforming growth factor subunit DMitogenEc0692714_m1PGF20Patched 2Tumor suppressorEc0692714_m1SOSTScherostinScherostinEc0703688_m1TGFATransforming growth factor bata 1Regulate proliferation	EPGN	Epithelial mitogen	Mitogen	 Ec06992859_m1
FGF5Fibroblast growth factor 5MitogenEc04686774_m1FGF18Fibroblast growth factor 18MitogenEc03248217_g1GDF6Growth differentiation factor 6Turnor suppressorEc03248217_g1GDF6Growth differentiation factor 6Turnor suppressorEc06964563_m1GL13GL1 family zinc finger 3Regulate proliferationEc069625512_m1HDCFHepatoma-derived growth factorMitogenEc07037751_m1HGFHepatonyte growth factorMitogenEc07037751_m1IGF1Insulin-like growth factorMitogenEc07037471_m1IGF2P5Insulin-like growth factor binding protein 5Mitogen bindingEc07037471_m1METMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc0248680_m1ICO10146270p16, Oyclin-dependent kinase 4 inhibitor BSenescence markerEc0703741_m1PCNAC-mycProto-oncogene, receptor tyrosine kinaseRegulate proliferationEc024924312_m1PDGFDPlatelet-derived growth factor suburit DMitogenEc09974312_m1PNAPoliberating protein A1Inhibits DNA synthesisEc0703741_m1PNAS100 calcium binding protein A1Inhibits proliferationEc0347073_g1SNAI2Sanal family transcriptonal repressor 2Anti-apoptoticEc0347073_g1SNAI2ScherostinPro-apoptoticEc0692482_m1TGFATransforming growth factor beta 3Regulate proliferationEc094918_m1TGFATransforming growth factor beta 3Regulate p	FGF1	Fibroblast growth factor 1	Mitogen	Ec01092738 m1
FGF18Fibroblac growth factor 18MitogenEc03248217_g1GDF6Growth differentiation factor 6Tumor suppressorEc07097112_m1GLB1Beta-galactosidaseSenescone markerEc0865438_m1GLB2GL1 family zin finger 3Regulate proliferationEc0825512_m1HDGFHepatom-derived growth factorMitogenEc07000751_m1HGFHepatom-derived growth factorMitogenEc07000054_m1IGF1Insulin-like growth factor binding protein 5MitogenEc0370226_m1LOC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescone markerEc07007511_m1METMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc08470236_m1DC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescone markerEc0700751_m1MCCc-mycPro-cyclingEc0700751_m1PCNAProliferating cell nuclear antigenIncrease DNA replicationEc08470236_m1PCRDPitatel-derived growth factor subunit DMitogenEc08470173_g1PHBProhibitinInhibits DNA synthesisEc07037611_m1PGFDPitatel-derived growth factor subunit DMitogenEc084701713_g1SNA12Sanal family transcriptional repressor 2Anti-apoptoticEc0862542_m1SNA1Stolo calcium binding protein A1Nitbits proliferationEc0862542_m1SNA12ScherostinTransforming growth factor beta 2Regulate proliferationEc0862547_m1TGFB3Transforming growth factor beta 3Regulate	FGF5	Fibroblast growth factor 5	Mitogen	Ec04656774 m1
GPGF6Growth differentiation factor 6Turor suppressorEc07097112_m1GLB1Beta-galactosidaseSenescence markerEc008954363_m1GLB3GLI family zinc finger 3Regulate proliferationEc00895512_m1HGFHepatocyte growth factorMitogenEc070307751_m1HGFHepatocyte growth factor 1Mitogen hiding protein 5Ec070307761_m1IGF1Insulin-like growth factor 1Mitogen hiding protein 5Ec070307761_m1IGF8Insulin-like growth factor binding protein 5Senescence markerEc070307471_mHMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc0262241_m1MYCc-mycPro-cyclingEc07007511_m1POGFDPlatelet-derived growth factor subunit DMitogenEc06827412_m1PDGFDPlatelet-derived growth factor subunit DMitogenEc06827412_m1PHBProhibitinInhibits DNA synthesisEc0703751_m1PGFDSecorsinInnor suppressorEc06825424_g1SNA12Solo calcum binding protein A1Inhibits proliferationEc06825424_g1SNA12ScherostinPro-apoptolicEc06825424_g1SNA12ScherostinPro-apoptolicEc06825424_g1SNA12ScherostinPro-apoptolicEc06825424_g1SNA12ScherostinPro-apoptolicEc06825424_g1SNA12ScherostinPro-apoptolicEc06825424_g1SNA12ScherostinPro-apoptolicEc06825424_g1SNA12ScherostinPro-a	FGF18	Fibroblast growth factor 18	Mitogen	Ec03248217 g1
GLB1Beta-galactosidaseSenescence markerEco08954363_m1GLB3GLI family zinc finger 3Regulate proliferationEco0825512_m1HDCFHepatoma-derived growth factorMitogenEc07000751_m1HGFHepatonyte growth factor 1MitogenEc07000764_m1IGF1Insulin-like growth factor 1Mitogen bindingEc03468689_m1LCC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescence markerEc07007771_mHMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc082541_m1VCCc-mycPro-cyclingEc07007711_m1PCNAProliferating cell nuclear antigenIncrease DNA replicationEc0682542_m1PCGFDPatelet-derived growth factor subunit DMitogenEc0682542_m1PCH2Patelet-derived growth factor subunit DMitogenEc0682542_m1PTCH2Patelet-derived growth factor subunit DInhibits DNA synthesisEc07007511_m1PTGH2Patched 2Turmor suppressorEc06825424_m1SIO0A1S100 aclium binding protein A1Inhibits proliferationEc0682542_m1SOSTScherostinPro-expotidicEc06976890_m1TGFA3Transforming growth factor beta 1Regulate proliferationEc06976890_m1TGFA3Transforming growth factor beta 1Regulate proliferationEc06976892_m1TGFA4Transforming growth factor beta 2Regulate proliferationEc0692692_m1TGFA3Transforming growth factor beta 3Regulate proliferationEc068254	GDF6	Growth differentiation factor 6	Tumor suppressor	Ec07097112 m1
GLI amily zinc finger 3Regulate proliferationEcoRe2512_m1HDGFHepatoma-derived growth factorMitogenEc0700054_m1HGFHepatocyte growth factor 1MitogenEc07000054_m1IGF1Insulin-like growth factor binding protein 5Mitogen bindingEc03468689_m1LOC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescence markerEc0700751_m1METMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc0822441_m1MYCc-mycPro-cyclingEc0707511_m1PORAProliferating cell nuclear antigenIncrease DNA replicationEc08974312_m1PDGFDPlatelet-derived growth factor subunit DMitogenEc08974312_m1PHBProhibitinInhibitor DNA synthesisEc0707511_m1PTCH2Patched 2Tumor suppressorEc08625424_g1SIO0A1S100 calcium binding protein A1Inhibits DNA synthesisEc0862537_m1SOSTScherostinPro-apoptoticEc0862537_m1TGFATansforming growth factor beta 1Regulate proliferationEc0862537_m1TGFATransforming growth factor beta 2Regulate proliferationEc08949183_m1TGFB2Transforming growth factor beta 3Regulate proliferationEc08470588_m1TMP2Metalopeptidase inhibitor 2Decrease proliferationEc03470588_m1TGFATransforming growth factor beta 3Regulate proliferationEc03470588_m1TGFB3Transforming growth factor beta 3Regulate proliferationEc03470588_m1	GLB1	Beta-galactosidase	Senescence marker	Ec06954363 m1
HOGFHepaton-derived growth factorMitogenEc07037751_m1HGFHepatocyte growth factorMitogenEc07000054_m1IGF1Insulin-like growth factor 1MitogenEc0348689_m1IGF5Insulin-like growth factor binding protein 5Mitogen bindingEc03470296_m1IGC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescence markerEc07037751_m1HMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc0822441_m1MYCc-mycProto-oncogene, receptor tyrosine kinaseRegulate proliferationEc0897312_m1PDGFDPoliferating cell nuclear antigenIncrease DNA replicationEc0897312_m1PHBProhibitinInhibits DNA synthesisEc07037751_m1PTCH2Patched 2Tumor suppressorEc08625424_g1S100A1S100 calcium binding protein A1Inhibits proliferationEc08625424_g1SOSTScherostinPro-apoptoticEc06825397_m1TGFATransforming growth factor alphaRegulate proliferationEc06949183_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc08625477_m1TGFB2Transforming growth factor beta 2Beculate proliferationEc03470588_m1TMP2Metalopeptidase inhibitor 2Decrease proliferationEc03470588_m1TGFB3Transforming growth factor beta 3Regulate proliferationEc03470588_m1TGFB4Transforming growth factor beta 3Regulate proliferationEc03470588_m1TMP2Metalopeptidase i	GLI3	GLI family zinc finger 3	Regulate proliferation	Ec06625512 m1
HereHereHereHereHereIGF1Insulin-like growth factor 1MitogenEc07000054_m1IGF1Insulin-like growth factor binding protein 5Mitogen bindingEc03468689_m1IGFBP5Insulin-like growth factor binding protein 5Mitogen bindingEc0707471_mHMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc0707511_m1MYCc-mycPro-cyclingEc0700511_m1POGPDPlatelet-derived growth factor subunit DMitogenEc06997142_m1PDGFDPlatelet-derived growth factor subunit DMitogenEc0699714_m1PHBProlibitinInhibits DNA synthesisEc07055990_m1PTCH2Patched 2Tumor suppressorEc06825424_g1S100 aclium binding protein A1Inhibits poliferationEc06925424_g1SNA2Snail family transcriptional repressor 2Anti-apoptoticEc06625397_m1SNA2SonsinPro-apoptoticEc06925424_g1SNA2Snail family transcriptional repressor 2Anti-apoptoticEc06925437_m1GFATransforming growth factor beta 1Regulate proliferationEc0692547_m1TGFATransforming growth factor beta 1Regulate proliferationEc0692547_m1TGFB1Transforming growth factor beta 3Regulate proliferationEc0682547_m1TGFB2Transforming growth factor beta 3Regulate proliferationEc0682547_m1TGFB3Transforming growth factor beta 3Regulate proliferationEc03470558_m1TMP2Metal	HDGF	Hepatoma-derived growth factor	Mitogen	Ec07037751 m1
IGF1Insulin-like growth factor 1MitogenE-03468689_m1IGFBP5Insulin-like growth factor binding protein 5Mitogen bindingE-03470296_m1LOC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescence markerE-07037471_mHMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationE-02622441_m1MYCc-mycProto-oncogene, receptor tyrosine kinaseRegulate proliferationE-02622441_m1MYCc-mycProto-oncogene, receptor tyrosine kinaseRegulate proliferationE-069974312_m1PCNAPoliferating cell nuclear antigenIncrease DNA replicationE-069974312_m1PDGFDPlatelet-derived growth factor subunit DMitogenE-06625424_g1PTCH2Patched 2Turmor suppressorE-06625424_g1S100 calcium binding protein A1Inhibits proliferationE-006625424_g1SNAI2Snail family transcriptional repressor 2Anti-apoptoticE-066926397_m1SOSTScherostinPro-apoptoticE-00692692_m1TGFATransforming growth factor beta 1Regulate proliferationE-006625477_m1TGFB1Transforming growth factor beta 3Regulate proliferationE-006625477_m1TGFB2Transforming growth factor beta 3Regulate proliferationE-006625477_m1TGFB3Transforming growth factor beta 3Regulate proliferationE-006625477_m1TGFB3Transforming growth factor beta 3Regulate proliferationE-0066253_m1TMP2Metallopeptidase inhibitor 2Decrease pro	HGF	Hepatocyte growth factor	Mitogen	Ec07000054 m1
InstrumeInstrumeInstrumeInstrumeIGFBP5InstrumeInstrumeEc03470296_m1LOC100146270p16, Cyclin-dependent kinase 4 inhibitor BSenescence markerEc07037471_mHMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc02622441_m1MYCc-mycPro-cyclingEc07007511_m1PCNAProliferating cell nuclear antigenIncrease DNA replicationEc06974312_m1PDGFDPlatelet-derived growth factor subunit DMitogenEc06997714_m1PHBProhibitinInhibits DNA synthesisEc07055990_m1PTCH2Patched 2Tumor suppressorEc06825424_g1S100A1S100 calcium binding protein A1Inhibits proliferationEc06972692_m1SNAI2Snail family transcriptional repressor 2Anti-apoptoticEc06825397_m1SOSTScherostinPro-apoptoticEc06827692_m1TGFA1Transforming growth factor alphaRegulate proliferationEc06972692_m1TGFB2Transforming growth factor beta 1Regulate proliferationEc0682167_m1TGFB3Transforming growth factor beta 2Regulate proliferationEc0682167_m1TGFB3Tumor protein 53Tumor propessorEc04347058_m1TPS3Tumor protein 53Tumor suppressorEc04347058_m1TPS3Beta-9curontidaseFindogenous controlEc0347058_m1TPS3Beta-9curontidaseEc03470648_m1Ec03470648_m1FDFABeta-9curontidaseEc03470648_m1Ec03470648_m1 <td>IGF1</td> <td>Insulin-like growth factor 1</td> <td>Mitogen</td> <td>Ec03468689 m1</td>	IGF1	Insulin-like growth factor 1	Mitogen	Ec03468689 m1
LCC100146270p16, Cyclin-dependent kinase inhibitor BSenescence markerEc07037471_mHMETMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEc02622441_m1MYCc-mycPro-cyclingEc07007511_m1PCNAProliferating cell nuclear antigenIncrease DNA replicationEc06997141_m1PDGFDPlatelet-derived growth factor subunit DMitogenEc06997714_m1PHBProhibitinInhibits DNA synthesisEc07055990_m1PTCH2Patched 2Tumor suppressorEc06625424_g1S10001S100 calcium binding protein A1Inhibits proliferationEc06625397_m1SOSTScherostinPro-apopticEc0689771_m1GFATiansforming growth factor alphaRegulate proliferationEc0689788_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc06892789_m1TGFATransforming growth factor alphaRegulate proliferationEc0689277_m1TGFB2Transforming growth factor beta 1Regulate proliferationEc0682477_m1TGFB3Transforming growth factor beta 2Regulate proliferationEc0847058_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc0347058_m1TPS3Tumor protein 53Tumor suppressorEc03470648_m1TPS4Vascular endothelial growth factor AMitogenEc03470648_m1PEGFDBeta-glucoronidaseEc0347058_m1Ec03470689_m1TMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470648_m1 <tr< td=""><td>IGFBP5</td><td>Insulin-like growth factor binding protein 5</td><td>Mitogen binding</td><td> Ec03470296_m1</td></tr<>	IGFBP5	Insulin-like growth factor binding protein 5	Mitogen binding	 Ec03470296_m1
METMET proto-oncogene, receptor tyrosine kinaseRegulate proliferationEco282241_m1MYCc-mycPro-cyclingEco7007511_m1PCNAProliferating cell nuclear antigenIncrease DNA replicationEco6974312_m1PDGFDPlatelet-derived growth factor subunit DMitogenEco6997714_m1PHBProhibitinInhibits DNA synthesisEco7055990_m1PTCH2Patched 2Tumor suppressorEco6625424_g1S100 calcium binding protein A1Inhibits proliferationEco6625397_m1SOSTScherostinPro-apoptoticEco6625397_m1SOSTScherostinPro-apoptoticEco6972692_m1TGFATransforming growth factor alphaRegulate proliferationEco69872692_m1TGFB1Transforming growth factor beta 1Regulate proliferationEco6625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEco6826477_m1TGFB3Transforming growth factor beta 3Regulate proliferationEco682613_m1TIMP2Metalopeptidase inhibitor 2Decrease proliferationEco347058_m1TPS3Tumor protein 53Tumor suppressorEco3470648_m1VEGFAVascular endothelial growth factor AAMitogenEco3470648_m1PES4Seta-2-microblobulinEndogenous controlEc03470630_m1TMP2Beta-2-microblobulinEndogenous controlEc034470630_m1PES4Beta-2-microblobulinEndogenous controlEc034470630_m1PES4Beta-2-microblobulinEndogenous control	LOC100146270	p16. Cvclin-dependent kinase 4 inhibitor B	Senescence marker	Ec07037471 mH
MYCc-mycPro-cyclingEc07007511_m1PCNAProliferating cell nuclear antigenIncrease DNA replicationEc06974312_m1PDGFDPlatelet-derived growth factor subunit DMitogenEc06997714_m1PHBProhibitinInhibits DNA synthesisEc07055990_m1PTCH2Patched 2Tumor suppressorEc06625424_g1S100 Alcium binding protein A1Inhibits proliferationEc0625397_m1SNAI2Snail family transcriptional repressor 2Anti-apoptoticEc06625397_m1SOSTScherostinPro-apoptoticEc0897682_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc08972682_m1TGFATransforming growth factor beta 1Regulate proliferationEc0625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc0842153_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470583_m1TPS3Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03470648_m1B2MBeta-2-microblobulinEndogenous controlEc03470683_m1GUSBBeta-glucoronidaseEndogenous controlEc03470783_g1	MET	MET proto-oncogene, receptor tyrosine kinase	Regulate proliferation	 Ec02622441 m1
PCNAProferating cell nuclear antigenIncrease DNA replicationEco697431_m1PDGFDPlatelet-derived growth factor subunit DMitogenEco697714_m1PHBProhibitinInhibits DNA synthesisEc07055990_m1PTCH2Patched 2Tumor suppressorEco6625424_g1S100A1S100 calcium binding protein A1Inhibits proliferationEco3470173_g1SNAI2Snail family transcriptional repressor 2Anti-apoptoticEco6972692_m1SOSTScherostinPro-apoptoticEco6972692_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEco6972692_m1TGFATransforming growth factor alphaRegulate proliferationEco682163_m1TGFB1Transforming growth factor beta 1Regulate proliferationEco082163_m1TGFB2Transforming growth factor beta 3Regulate proliferationEco082163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEco03470658_m1TPS3Tumor protein 53Tumor suppressorEco0347677_m1PS4Vascular endotheila growth factor AMitogenEco0346787_m1B2MBeta-2-microblobulinEndogenous controlEco3476879_m1B2MBeta-glucoronidaseEco0346869_m1Eco346869_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc0347683_m1	MYC	c-myc	Pro-cycling	 Ec07007511 m1
PDGFDPlatelet-derived growth factor subunit DMitogenEc06997714_m1PHBProhibitinInhibits DNA synthesisEc07055990_m1PTCH2Patched 2Tumor suppressorEc06625424_g1S100A1S100 calcium binding protein A1Inhibits proliferationEc08470173_g1SNAl2Snail family transcriptional repressor 2Anti-apoptoticEc06625397_m1SOSTScherostinPro-apoptoticEc066972692_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc06949183_m1TGFATransforming growth factor alphaRegulate proliferationEc06825477_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc0682163_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc0682163_m1TMP2Metallopeptidase inhibitor 2Decrease proliferationEc043470558_m1TMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03470648_m1B2MBeta-2-microblobulinEndogenous controlEc03467879_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	PCNA	Proliferating cell nuclear antigen	Increase DNA replication	 Ec06974312_m1
PHBProhibitinInhibits DNA synthesisEc0705599(m1)PTCH2Patched 2Tumor suppressorEc06625424_g1S100A1S100 calcium binding protein A1Inhibits proliferationEc03470173_g1SNAI2Snail family transcriptional repressor 2Anti-apoptoticEc07036868_m1SOSTScherostinPro-apoptoticEc00972692_m1TERTTelomerase reverse transcriptaseSuburit of telomeraseEc06972692_m1TGFATransforming growth factor alphaRegulate proliferationEc06825477_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc07074189_g1TGFB2Transforming growth factor beta 2Regulate proliferationEc0082163_m1TGFB3Transforming growth factor beta 3Regulate proliferationEc03470558_m1TMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03470879_m1B2MBeta-2-microblobulinEndogenous controlEc03468699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	PDGFD	Platelet-derived growth factor subunit D	Mitogen	Ec06997714 m1
PTCH2Patched 2Tumor suppressorEco6625424_g1S100A1S100 calcium binding protein A1Inhibits proliferationEc06625397_m1SNAl2Snail family transcriptional repressor 2Anti-apoptoticEc06625397_m1SOSTScherostinPro-apoptoticEc06972692_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc066972692_m1TGFATransforming growth factor alphaRegulate proliferationEc06625477_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc06625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc0682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03470879_m1B2MBeta-2-microblobulinEndogenous controlEc0347083_m1GUSBBeta-glucoronidaseEndogenous controlEc0347083_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	PHB	Prohibitin	Inhibits DNA synthesis	Ec07055990 m1
S100A1S100 calcium binding protein A1Inhibits proliferationEc03470173_g1SNAl2Snail family transcriptional repressor 2Anti-apoptoticEc06625397_m1SOSTScherostinPro-apoptoticEc07036868_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc06972692_m1TGFATransforming growth factor alphaRegulate proliferationEc06625477_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc06625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc00682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03470630_m1GUSBReta-glucoronidaseEndogenous controlEc03470630_m1RPLPORibosomal protein lateral stalk subunit POEndogenous controlEc0347733_g1	PTCH2	Patched 2	Tumor suppressor	Ec06625424 g1
SNAI2Snail family transcriptional repressor 2Anti-apoptoticEc06625397_m1SOSTScherostinPro-apoptoticEc07036868_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc06972692_m1TGFATransforming growth factor alphaRegulate proliferationEc06625477_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc06625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc07074189_g1TGFB3Transforming growth factor beta 3Regulate proliferationEc0082163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03470630_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLPORibosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	S100A1	S100 calcium binding protein A1	Inhibits proliferation	Ec03470173 g1
SOSTScherostinPro-apoptoticEc07036868_m1TERTTelomerase reverse transcriptaseSubunit of telomeraseEc06972692_m1TGFATransforming growth factor alphaRegulate proliferationEc06949183_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc06625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc0082163_m1TGFB3Transforming growth factor beta 3Regulate proliferationEc03470558_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2microblobulinEndogenous controlEc03470630_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLPORibosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	SNAI2	Snail family transcriptional repressor 2	Anti-apoptotic	Ec06625397 m1
TERTTelomerase reverse transcriptaseSubunit of telomeraseEc06972692_m1TGFATransforming growth factor alphaRegulate proliferationEc06949183_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc060525477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc07074189_g1TGFB3Transforming growth factor beta 3Regulate proliferationEc00682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03470630_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLPORibosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	SOST	Scherostin	Pro-apoptotic	Ec07036868 m1
TGFATransforming growth factor alphaRegulate proliferationEc06949183_m1TGFB1Transforming growth factor beta 1Regulate proliferationEc06625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc07074189_g1TGFB3Transforming growth factor beta 3Regulate proliferationEc00682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03470630_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLPORibosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	TERT	Telomerase reverse transcriptase	Subunit of telomerase	Ec06972692 m1
TGFB1Transforming growth factor beta 1Regulate proliferationEc06625477_m1TGFB2Transforming growth factor beta 2Regulate proliferationEc07074189_g1TGFB3Transforming growth factor beta 3Regulate proliferationEc00682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03470630_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	TGFA	Transforming growth factor alpha	Regulate proliferation	Ec06949183 m1
TGFB2Transforming growth factor beta 2Regulate proliferationEc07074189_g1TGFB3Transforming growth factor beta 3Regulate proliferationEc00682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03468699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	TGFB1	Transforming growth factor beta 1	Regulate proliferation	Ec06625477 m1
TGFB3Transforming growth factor beta 3Regulate proliferationEc00682163_m1TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03466699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit POEndogenous controlEc04947733_g1	TGFB2	Transforming growth factor beta 2	Regulate proliferation	Ec07074189 g1
TIMP2Metallopeptidase inhibitor 2Decrease proliferationEc03470558_m1TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03468699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	TGFB3	Transforming growth factor beta 3	Regulate proliferation	Ec00682163 m1
TP53Tumor protein 53Tumor suppressorEc03470648_m1VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03468699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	TIMP2	Metallopeptidase inhibitor 2	Decrease proliferation	Ec03470558 m1
VEGFAVascular endothelial growth factor AMitogenEc03467879_m1B2MBeta-2-microblobulinEndogenous controlEc03468699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	TP53	Tumor protein 53	Tumor suppressor	Ec03470648 m1
B2MBeta-2-microblobulinEndogenous controlEc03468699_m1GUSBBeta-glucoronidaseEndogenous controlEc03470630_m1RPLP0Ribosomal protein lateral stalk subunit P0Endogenous controlEc04947733_g1	VEGFA	Vascular endothelial growth factor A	Mitogen	Ec03467879 m1
GUSB Beta-glucoronidase Endogenous control Ec03470630_m1   RPLP0 Ribosomal protein lateral stalk subunit P0 Endogenous control Ec04947733_g1	B2M	Beta-2-microblobulin	Endogenous control	Ec03468699 m1
RPLP0 Ribosomal protein lateral stalk subunit P0 Endogenous control Ec04947733_g1	GUSB	Beta-glucoronidase	Endogenous control	 Ec03470630 m1
	RPLP0	- Ribosomal protein lateral stalk subunit P0	Endogenous control	_ Ec04947733_g1

\*NIH, Genetics Home Reference (https://ghr.nlm.nih.gov/gene/).



adipose tissue (AT)-derived mesenchymal stem cells (MSC) and dermal fibroblasts (DF) from horses in five different age groups (n = 4, N = 60) after 24 h of labeling with 8  $\mu$ M EdU. Age groups within the same cell type not labeled with the same letter are significantly different from each other (p < 0.05). d., days old; mo., months old; yo., years old.

group reached a cellular proliferation of 100% (**Figure 1**). Alexa 594 staining was detected in all wells pulsed with EdU, together with DAPI staining.

## **Gene Expression**

Out of the 47 targeted gene loci related to proliferation and aging, steady state mRNA levels were affected by donor age in four biomarkers (9%) for AT-MSCs, 17 biomarkers (36%) for BM-MSCs, and 15 biomarkers (32%) for DFs. Differentially expressed genes as a function of donor age are shown in **Table 3** and in a Venn-diagram in **Figure 4** where intersections are visualized.

Multiple growth factors (FGF1, FGF18, GDF6, PDGFD, TGFA, and VEGFA) were up-regulated in BM-MSCs from old horses compared to young horses. On the other hand, fibroblast growth factor 5 (FGF5) was found at lower levels in BM-MSCs from geriatric horses compared to newborns (p = 0.02), and in higher levels in AT-MSCs compared to BM-MSCs (p =0.008). Cell cycle regulators cyclin D (CCND1) and proliferating cell nuclear antigen (PCNA) were not significantly affected by donor age in either of the two stem cell types, but were downregulated in DFs from geriatric horses (p = 0.01 and p < 0.0001, respectively). In general, cyclin D was found at higher levels in BM-MSCs compared to AT-MSCs and DFs (p < 0.003), whereas PCNA was found at higher levels in DFs compared to AT-MSCs (p < 0.0001) and BM-MSCs (p = 0.0004). For positive regulators of cell proliferation colony stimulating factor 2 (CSF2) was upregulated in BM-MSCs from geriatric horses compared to all other age groups (p < 0.0005), whereas GLI family zinc finger 3 (GLI3) was down-regulated in geriatric BM-MSCs ( $p \le 0.02$ ) (Figure 5, Table 3).

For BM-MSCs, expression of the senescence marker  $\beta$ -galactosidase (GLB1) was significantly higher in geriatric

horses compared to newborns (p = 0.002), yearlings (p = 0.02), and adult horses (p = 0.03), and in middle-aged compared to newborn horses (p = 0.01). GLB1 was not significantly affected by donor age in AT-MSCs or DFs. Higher GLB1 expression was seen in BM-MSCs compared to AT-MSCs and DFs in geriatric horses (p = 0.001 and p = 0.0003, respectively) (**Figure 5**, **Table 3**). Steady state mRNA levels of aquaporin (AQP1), which encodes a membrane-associated water channel protein, were low relative to the positive control sample and changed as a function of age in all three cell types, increasing in older horses for BM-MSCs and DFs while decreasing in AT-MSCs.

As shown in **Figure 6**, the tumor suppressors p16 (LOC100146270) and p21 (CDKN1A) were both up-regulated in geriatric BM-MSCs compared to newborn horses (p = 0.01 and p = 0.02, respectively). Moreover, p16 and p21 showed higher levels in BM-MSCs than in AT-MSCs ( $p \le 0.02$ ) or DFs ( $p \le 0.002$ ). For AT-MSCs, the tumor suppressor p53 (TP53) had higher expression in geriatric horses compared to newborn (p = 0.002) or yearlings (p = 0.008). The pro-apoptotic genes caspase 3 (CASP3) and caspase 8 (CASP8) were up-regulated in BM-MSCs from older horses ( $p \le 0.003$ ).

## DISCUSSION

Results from the present study show that *in vitro* proliferation percentages of plastic-adherent equine BM- and AT-MSCs were stable through adult ages, but decreased in samples collected from geriatric donors. These findings support the hypothesis generally consistent with Alicka et al., who showed a shorter population doubling time in AT-MSCs from horses below 5 years of age compared to horses above 15 years of age (18). Additionally, Vidal et al. reported no difference in proliferative





capacity of BM-MSCs from horses below 5 years of age (16). Schröck et al. reported a heterogeneous population of equine BM-MSCs with a decreasing population of cells with maximum proliferation speed with increasing donor age (20). Schröck's study, however, had a limited study population and was not designed to provide kinetics or thresholds with regards to donor age. The current study extends our understanding in horses by analyzing additional age groups, and interestingly shows that difference in equine BM- and AT-MSC cellular proliferation were only seen in pair-wise comparisons involving geriatric horses. No difference in cellular proliferation rates were observed in MSCs from horses below 18 years of age. This finding is broadly



**TABLE 3** | Differentially expressed genes related to cellular proliferation as a function of donor age within adipose tissue- and bone marrow derived mesenchymal stem cells and dermal fibroblasts from horses in five different age groups (n = 4, N = 60) after one-way ANOVA statistical analysis when significance was set to p < 0.05.

Cell type	Gene function							
	Senescence marker	Cell cycling	Growth factor	Anti-apoptotic	Tumor suppressor	Apoptotic		
AT-MSC		Down-regulated	Down-regulated HGF		Up-regulated TP53	Up-regulated SOST		
BM-MSC	Up-regulated GLB1	Up-regulated AQP1 CSF2 CTGF Down-regulated GLI3	Up-regulated FGF1 FGF18 PDGFD TGFA VEGFA Down-regulated FGF5		Up-regulated CDKN1A GDF6 LOC100146270	Up-regulated CASP3 CASP8 SOST		
DF		Up-regulated AQP1 COMP Down-regulated CCND1 PCNA	Up-regulated FGF18 IGFBP5 Down-regulated EPGN HGF PDGFD	Up-regulated CLU Down-regulated BCL2	Up-regulated GDF6 Down-regulated LOC100146270 TP53	Down-regulated CASP3		

\*All listed genes were significantly affected by donor age within the given cell type.

<sup>†</sup>AT, adipose tissue; BM, bone marrow; MSC, mesenchymal stem cells; DF, dermal fibroblasts. Red marked genes indicate an up-regulation in gene expression with increasing donor age. Blue marked genes indicate a down-regulation in gene expression with increasing donor age.



consistent with the gene expression data, where the majority of age-related changes in steady state mRNA levels were seen in comparisons involving geriatric horses. Decline in proliferative capacity with increasing donor age is similar to previous reports in other species (12–15). However, the pair-wise comparisons from horses stand in contrast with other species where differences in proliferation were more progressive and reported already in 8-year-old monkeys (12) and in humans above 40 years of age (32).

Cellular proliferation of human AT-MSCs have previously been reported to be less prominently affected by donor age compared to BM-MSCs (33). This was not observed in the present study, where equine BM- and AT-MSCs showed similar cellular proliferation in all age groups and were equally affected by donor age. Together, this may heighten the relevance of BM-MSCs for autologous treatments, as they have shown a higher therapeutic potential for cartilage and bone injuries (6, 34, 35). On the other hand, less age related gene expression changes were seen in AT-MSCs with the selected biomarker panel compared to BM-MSCs or DFs. It is likely that the cells have other age-related expression changes outside the selected biomarker panel as the phenotypic cellular proliferation was equal for AT- and BM-MSCs across age groups.

Our finding that DFs have generally higher cellular proliferation compared to BM-and AT-MSCs is supported by previous studies where DFs have been shown to be highly sensitive to mitogenic stimuli and readily expand in adherent monolayer cultures (19). The decrease in cellular proliferation with increasing donor age for all three cell types is broadly consistent with a general decline in the proliferative capacity of equine primary cells isolated from donor horses with increasing age.

Molecular mechanisms responsible for the observed decrease in equine cellular proliferation in samples collected from geriatric donors could potentially include a decrease in growth factors and their receptors, a decrease in pro-cycling molecules, and/or an increase in cell cycle arrest genes and apoptotic factors. Similar to previous studies, our data demonstrated that the tumor suppressor genes p53, p21 and p16 were up-regulated in MSCs from old donors (12, 14, 18). p16 and p21 inhibit DNA replication by inhibiting cyclin dependent kinases and are considered markers of senescence (36). p53 is a transcription factor for p21 and can induce transcription of apoptosis-associated genes like BAX, which leads to activation of CASP3, another apoptotic factor (37). Interestingly, increased p53 expression in old AT-MSCs did not result in detectable up-regulation of p21 or BAX in the geriatric AT-MSCs, which might be due to a lack of phosphorylation of p53 and a resulting rapid degradation. An increase in the apoptotic pro-factor CASP8 can also cause up-regulation of CASP3 (37), possibly explaining why both CASP8 and CASP3 were up-regulated in BM-MSCs from geriatric horses.

Taken together, the age related up-regulation of tumor suppressors and apoptotic factors could explain the lower cellular proliferation seen in aged horses. This supports a model of more cells being growth arrested rather than progressing through the S-phase of the cell cycle and incorporating EdU, which is consistent with previous studies where increased G1/G0-arrest and prolonged time in S-phase have been reported in MSCs from aged donors (12, 18).

GLB1 activity is an additional marker of senescence, with higher gene expression in senescent cells being reported (38). In BM-MSCs, an age related increase in GLB1 expression was seen in geriatric horses corresponding well with previous studies showing accelerated cellular senescence with increasing donor age (15, 18).

Interestingly, multiple growth factors actually displayed higher steady state mRNA levels in BM-MSCs from geriatric horses. Age-dependent growth factor results are variable in previous rodent studies (13, 39, 40), but the current data suggests that the decreased proliferation observed in BM-MSCs from geriatric horses may be more due to an upregulation in tumor-suppressors and apoptotic factors than a decrease in growth factors or pro-cycling factors. It is moreover possible that aged BM-MSCs are less responsive to secreted growth factors, consistent with studies investigating relationships between growth factor activity and inflammation in aging (41).

Potential age-dependent differences in cellular differentiation potential, as well as production of paracrine factors to influence the patient's immune response or endogenous progenitor cells relevant to cell-therapy application in horses were not investigated in the current experiments, but will be important parameters to assess in future studies. Another parameter not addressed was potential gender differences in the proliferation of equine MSCs as a function of age.



Primary cells were collected from female and male horses in each age group, but not with even distribution (**Table 1**). A relevant study in mice comparing *in vitro* MSC proliferation as a function of gender did not observe any differences (42). All donor horses in the study were in apparent good health for their age with a few exceptions in older animals (**Table 1**). Certain systemic health parameters could affect cell biology parameters including proliferation. The impact of specific systemic illnesses on MSCs, as in the case of the 25-year-old lymphoma horse in the current sample set, will require additional research. Primary cell lines in the different age groups did consist of both paired and non-paired samples. Ideally, the study would have been conducted solely with paired samples as inter-animal variation

is an important consideration (43). To control for this factor, the data were treated statistically as non-paired and horse number was added to the statistical models.

Evidence for trilineage differentiation potential is apparent in equine BM- and AT-MSCs isolated using the techniques applied in this study (data not shown). Unfortunately, uniform parameters to validate "stemness" of equine MSCs with cell surface molecular markers has not been established (44) and relevant antibodies for equine cell isolates remain limited (45–47).

In conclusion, all cells independent of age group were able to be expanded to passage 4, but the proliferation of plastic adherent equine BM- and AT-MSCs declined significantly in the geriatric



age group. High *in vitro* cellular proliferation was seen in BMand AT-MSCs from horses below 18 years of age. The cellular proliferation of BM- and AT-MSCs was similar in all age groups and they were equally affected by donor age. Underlying gene expression changes related to proliferation and aging showed a primary up-regulation in tumor suppressors, apoptotic genes, and multiple growth factors in MSCs from old horses, and a down-regulation of some pro-cycling genes depending on cell type. MSC differentiation potential, as well as the capacity to mediate inflammatory processes and other paracrine functions will be important parameters to consider with further research on donor age as a variable in cell-based therapies for horses.

# DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

# **ETHICS STATEMENT**

The animal study was reviewed and approved by the ethical guidelines of animal research at the University of Copenhagen and the University of Kentucky. Written informed consent was obtained from the owners for the participation of their animals in this study.

# AUTHOR CONTRIBUTIONS

JB, JM, and LB were responsible for the study design, harvest of cells, and for obtaining funding. Acquisition of data, data analyses

and interpretation, and writing the first draft of the manuscript was done by JB. All authors were involved in critical revision of the manuscript and final approval.

# FUNDING

This study was partly funded by the Independent Research Fund Denmark (NIH 133500133B), Hesteafgiftsfonden, Foreningen KUSTOS af 1881, and The Lourie Family Foundation. Funding organizations had no role in study design, data collection, analysis and interpretation, manuscript writing or the decision to submit the manuscript for publication.

# ACKNOWLEDGMENTS

The authors wish to thank Matthew Rutledge, Eva Loveland, Dr. Arnold Stromberg, and Dr. Ashley Steuer for help with statistical analyses; Dr. Emma Adam and Dr. Parvathy Thampi for efforts in generating some of the primary cell lines; and Maria Rhod, Simone Buchardt, Emily Melcher, Bianca Ruspi, and Jonas Bagge for laboratory assistance.

# SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets. 2020.602403/full#supplementary-material

 $\label{eq:supplementary Table 1 | Table with Ct and Ln(RQ) data used for statistical analysis and the heatmap.$ 

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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