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Temperol dynamics of gene activity in neonatal, senescent and adult dermal fibroblasts in response to TGF- β

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Skin is a complex tissue composed of the dermal and epidermal layers. The dermis contains an extracellular matrix (ECM) that is maintained by fibroblasts in response to biochemical cues such as TGF- β stimulation. With age, the composition of the ECM changes, rendering aged skin less adept at performing its functions. While some of the differences between young and old dermis are known, a more complete understanding would help us in efforts to restore function to old tissue. Here, we have performed a high throughput qPCR experiment to measure the dynamics of 72 transcripts relevant to skin ageing in neonatal, adult and irradiation-induced senescent dermal fibroblasts in response to TGF- β . Our results show that clear differences exist between the different fibroblasts. To facilitate exploration of these data, a web application has been built to host and interactively visualise the data. Collectively this work furthers our understanding of how age and senescence affects the dermal fibroblasts, which is an essential step for understanding why differences exist and how to rectify them.

KEY WORDS

Skin, Ageing, Dermal fibroblasts, High throughput qPCR, TGF- β

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1 | INTRODUCTION

Ageing can broadly be described as the progressive deterioration of biological function with time. While not itself a disease, ageing is the biggest risk factor for neurodegenerative, cardiovascular and cancerous diseases ([Niccoli and Partridge, 2012](#)). With an increasingly ageing population it is important to study how tissues age to better understand how we might develop the therapeutic potential to promote healthy ageing.

Skin is the largest organ in the human body and it performs numerous functions beyond that of a barrier to environmental stresses. For example, skin is involved in sensory perception, thermoregulation and immunosurveillance. Like other tissues, skin is subject to intrinsic ageing, where the ability of skin to perform its functions is diminished. Phenotypically, old skin is dry, rough and itchy with uneven pigmentation, a reduced capacity for wound healing, wrinkles and impaired collagen and elastin networks. Old skin has diminished hair growth and sebaceous gland function, flatter dermal papillae, reduced melanocyte concentrations and less cellular turnover, compared with young skin. The ageing of skin is the most visible aspect of ageing and the resulting phenotypic changes have an important impact both on physiological and psychological well-being ([Blume-Peytavi et al., 2016](#); [Farage et al., 2009](#)). A greater understanding of how skin ageing manifests would benefit the development of therapeutic and cosmetic interventions to promote healthy skin ageing.

Skin is a multi-layered tissue composed of an outer epidermis and a underlying dermis. The epidermis is comprised mainly of keratinocytes which are avascular and gradually differentiate as they harden and progress outwards towards the skin surface. Beneath the epidermis lies the dermal-epidermal junction which is a thin basement membrane that enables communication between the dermis and epidermis. The dermis is a vascular, cellular sparse tissue composed mostly of a fibrous connective tissue known as the dermal extracellular matrix (ECM). Dermal tissue is essential for skin integrity by providing structural integrity and nourishing support for the epidermis ([Lu et al., 2011](#)).

Much like other tissues, an essential aspect of dermal ECM biology is that function arises as a consequence of structure. Dermal fibroblasts are responsible for synthesising ECM components as well as proteins which degrade them. Fibroblasts respond to biochemical cues such as stimulation by TGF- β or TNF- α , which induce changes in fibroblast secretory. In age, the composition of the dermal microenvironment is different from that of young tissue. Fibroblasts in young tissue reside in an environment under mechanical tension which arises from physical binding to the ECM. With age, collagen fibrils become increasingly fragmented and fewer in number as a result from decreased production and enhanced degradation by matrix metalloproteinases (MMPs), such as MMP1 ([?Quan et al., 2015](#); [Fisher et al., 2009](#); [Varani et al., 2006](#)). Consequently, fibroblasts in an aged environment have less physical associations with the dermis, inducing fibroblasts to take on a globular morphology. Globular fibroblasts exist under different mechanical tensions compared to appropriately 'spread out' fibroblasts and are less able to maintain the dermal ECM because of the different secretory profile. Consequently, ECM damage accumulates and results in a positive feedback cycle where skin deteriorates ([?Cole et al., 2018](#); [Quan et al., 2015](#); [Fisher et al., 2009](#); [Varani et al., 2006](#)).

The aged dermal environment contains an increasing population of senescent dermal fibroblasts. Cells have a limited replicative potential and when they can no longer proliferate they undergo replicative senescence. Importantly, senescent cells remain metabolically active and can interfere with normal physiology by secreting proteins into the ECM ([Toutfaire et al., 2017](#)). The collective profile of proteins that are secreted are known as the age-associated secretory profile or SASP (ref).

TGF- β signalling is essential for ECM homeostasis because it controls the production of many ECM components including type 1 collagen ([Varani et al., 2006](#); [Varga et al., 1987](#)), fibronectin (?), elastin (?) and presumably many other ECM components. Further, several reports have suggested that TGF- β also transcriptionally represses genes for proteases which degrade the ECM ([White et al., 2000](#); [Yuan and Varga, 2001](#); [Edwards et al., 1996](#)). In age, are

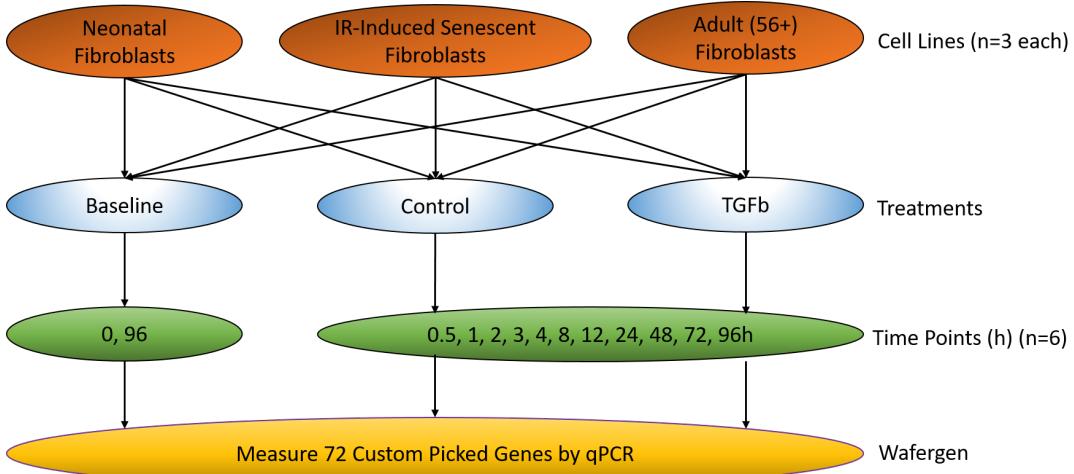


FIGURE 1 Graphical depiction of experimental design

TABLE 1 Table summarising the number of genes per comparison that were differentially expressed with a (FDR corrected) p-value <0.001 in over 60% of LIMMA analyses per group.

Comparison	Treatment	
	Control	TGF- β
Adult Vs neonatal	16	46
Senescent Vs neonatal	16	30
Neonatal Vs neonatal	7	28
Adult Vs adult	17	37
Senescent Vs senescent	10	25

alterations in the fibroblast TGF- β network contribute towards the ageing dermal phenotype. Notably, loss of Smad3 (Purohit et al., 2016) and reduced CTGF levels in aged fibroblasts have been proposed as mechanisms contributing towards reduced collagen levels in age.

In this study, we have conducted a high throughput qPCR experiment designed to study transcriptional differences between neonatal, adult and irradiation-induced senescent fibroblasts. Fibroblasts were treated with TGF- β or a negative control over time to compare the dynamics of gene activity between cell lines. We discuss some of these differences and provide an interactive web interface into the data for readers to explore the dataset.

2 | RESULTS

To identify some of the differences between neonatal, irradiation-induced senescent and adult fibroblasts, the activity of 72 genes was measured over 96h in 3 replicates of each cell group using high throughput qPCR. These measurements were taken both in response to 5ng mL⁻¹ TGF- β reconstituted in 10mM citric acid or 0.1% BSA in 10mM citric

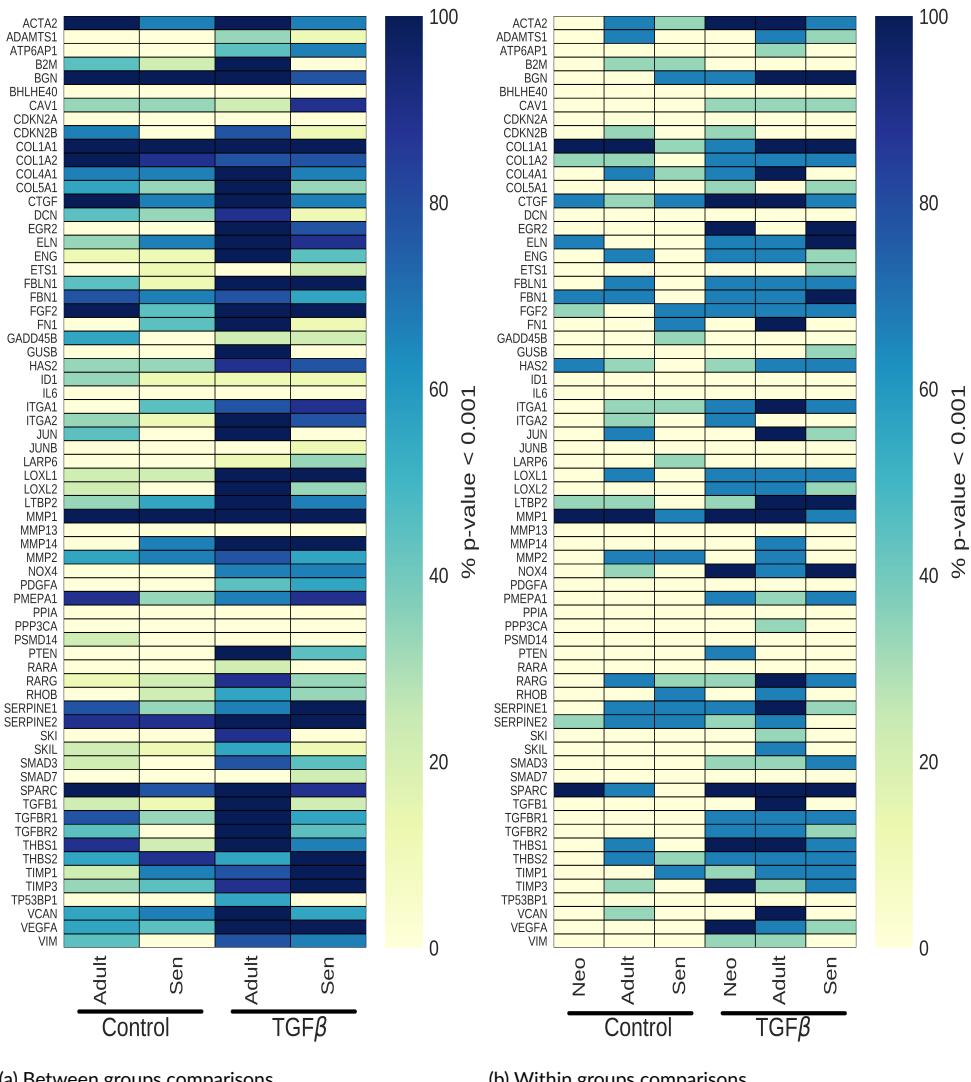


FIGURE 2 Differential expression analysis for (a) between groups comparisons and (b) within groups comparisons. Colours indicate the percentage of the time of all combinations (9 for between groups and 3 for within groups) of LIMMA analysis conducted that a time series was differentially expressed with a FDR corrected p-value >0.001.

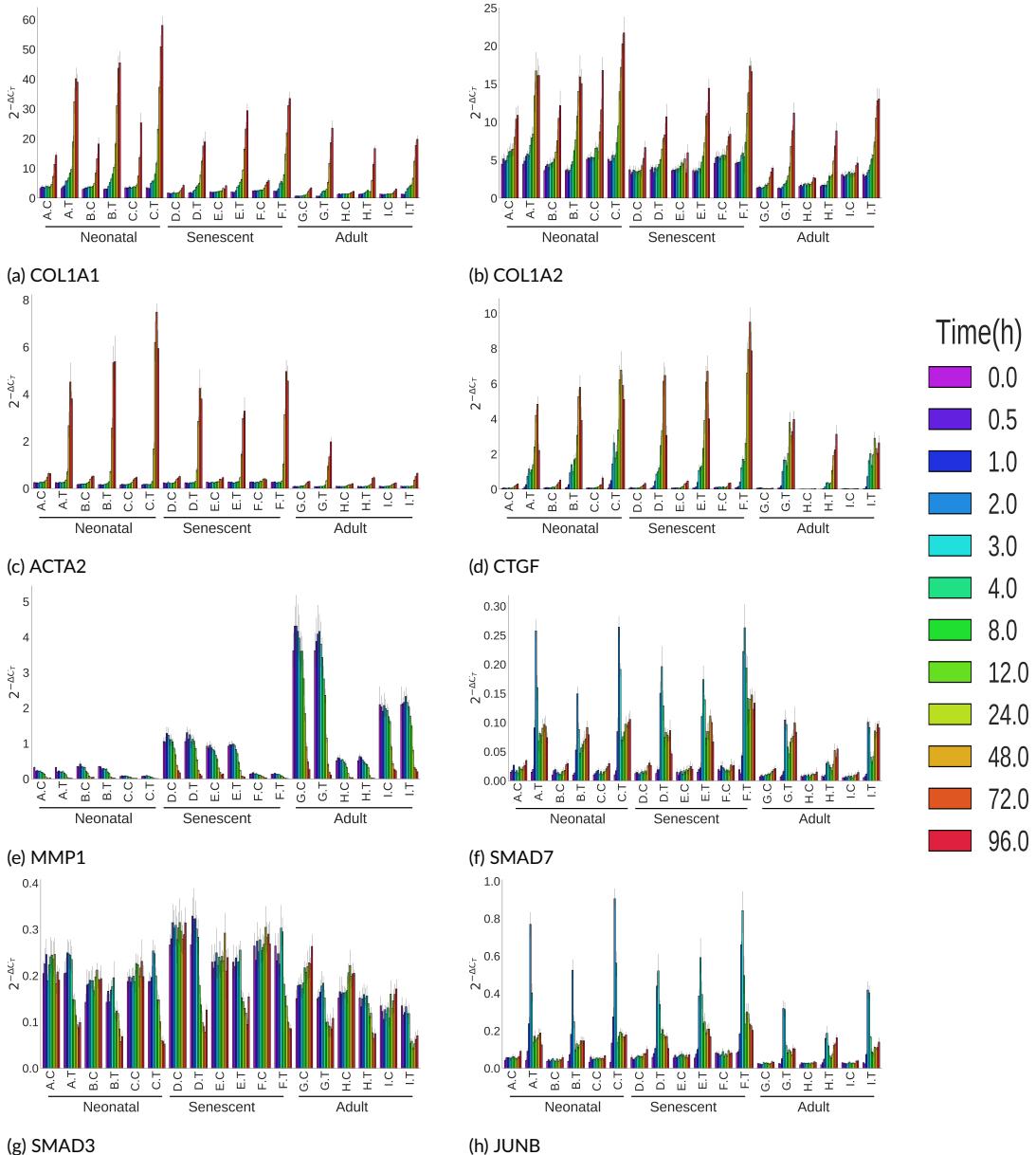


FIGURE 3 Bar graphs showing control (C) and TGF- β (T) treated time series datasets for each cell line (labelled A-I as described in the methods). Error bars represent standard error of the mean and each condition was repeated 6 times.

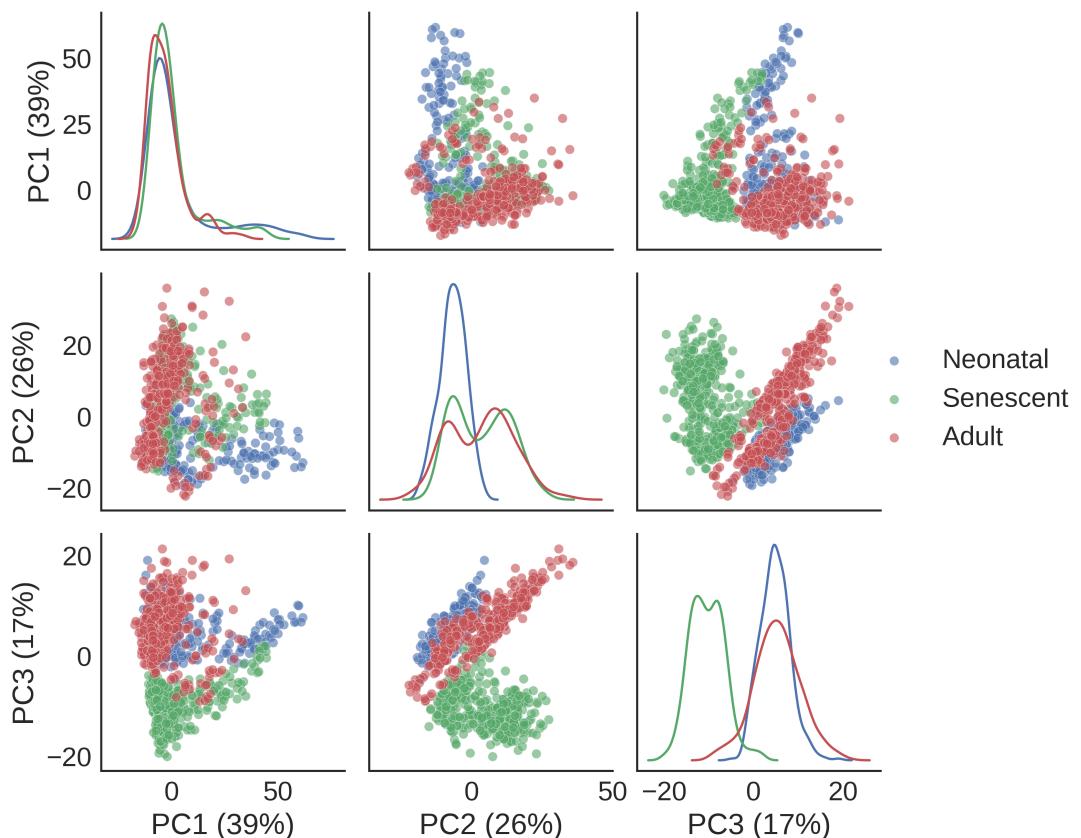


FIGURE 4 Scatter matrix of first three principle components coloured by cell line. Kernel density plots along the diagonal represent the distribution of the relevant principle component.

acid as a negative control. The genes measured were manually selected based on a literature search to be relevant to ECM biology or TGF- β signalling. The design of this experiment is depicted in (Figure 1). Three out of 72 primers (IL1A, IL1B and FOSB) were either unreliable or undetectable and were excluded from further analysis. As a first step in data analysis a principal component analysis (PCA) was conducted to gain confidence in the measurements. The PCA was produced so that each point on the PCA represents one of the 1296 samples. The closer together points lie on the PCA the more similar they are. Therefore it is possible to both positively and negatively assert whether the cells behave as expected. To highlight these experimental factors the same PCA was plotted 5 times, coloured by different experimental factors, namely by cell line (Figure 4), cell ID (Figure 6), time points (Figure 8), treatment (Figure 7) and by biological replicates (Figure 9). Because the PCA coloured by cell lines, cell IDs, time points and treatments all display clustering while biological replicates do not, this PCA indicates that the data collected likely represent the underlying biology well. A scree plot is presented in Figure 5 and indicates that the first three principal components account for >80% of variance.

Following the PCA, a differential expression analysis was conducted to investigate whether each measured gene was statistically different: 1) between in adult or senescent cell lines compared to neonatal cell lines or 2) within

each cell group. The R package LIMMA was used for differential expression analysis. Specifically, because our data is dynamic, LIMMA was configured to evaluate whether two full time series objects were different from each other (Ritchie et al., 2015; Smyth, 2004, 2005). Since we have data from 3 cell lines per cell line group, a comparison between cell lines G and A is just as valid as cell lines H and A, to compare adult and neonatal cells (for example). To make use of the available data, all combinations of cell line were compared using LIMMA, as explained in ???. A total of 10 differential expression analyses were conducted, 5 each to compare TGF- β or negative control time series, two of which were between groups comparisons and three within groups comparisons. A gene was considered differentially expressed if the FDR corrected p-value was less than 0.001. Since the between groups comparisons totalled 9 LIMMA analyses per comparison and the within groups comparisons totalled 3, an overview of the differential expression analysis is presented in Table 1 by counting the number of genes which were differentially expressed in >60% of LIMMA analyses per comparison. The differential expression analysis is expressed as a percentage in Figure 2 and shows that the comparison between TGF- β treated adult and neonatal cell lines were the most different of all the comparisons, followed by the comparison within TGF- β treated adult cell lines. The comparison within control treated neonatal cell lines were the least different. Moreover, in all cases, the TGF- β time series had more differentially expressed genes than the control time series.

Figure 3 shows a selection of the genes measured in this experiment. For a pdf document containing similar but full sized graphs for each measured gene, please see supplementary file 1.

Collagen 1A1 and 1A2 were produced as a consequence of time in culture Figure 3a and ?? and were strongly induced by TGF- β . Neonatal cell lines produced more collagens than both adult and senescent cell lines.

ACTA2 (α -SMA) and CTGF were both among the most strongly induced of the genes measured. TGF- β strongly induced both ACTA2 Figure 3c and CTGF Figure 3d in all cell lines but the induction was considerably weaker in adult cell lines (Figure 3c).

Senescent cell lines produced more MMP1 than neonatal cell lines while adult cells variably produced more MMP1 than both neonatal and senescent cell lines Figure 3e. The time series profile in all cell lines were similar in shape, but not in magnitude and TGF- β did not modulate the transcription of MMP1.

Smad7 is present in very small quantities without the presence of TGF- β while TGF- β treatment dramatically and transiently induced Smad7 transcription in all cell lines (Figure 3f). The peak magnitude is similar between senescent and neonatal cell lines but blunted in adult cell lines.

There is no describable pattern in Smad3 transcription under the control time series. However, in all cell lines tested, TGF- β induced a reduction in Smad3 production between after 8h of TGF- β stimulation.

Like Smad7, JunB transcription remains unchanged in the control condition but is strongly induced by TGF- β , peaking within 2h (Figure 3h). The amount of JunB produced is lower in the adult than in the neonatal cell lines.

The data accumulated in this experiment is available for viewing and download at <http://cwelsh2.pythonanywhere.com/>. The purpose of this website is to enable readers to interactively explore this dataset with minimal effort. Options are available for comparing different genes, cell lines, treatments and time points. Data can be viewed as a data table or as line plots and a 3D interactive PCA is also available for exploration.

Discussion

In aged skin, the composition of the dermal ECM changes resulting in thinner, weaker and less elastic skin (Farage et al., 2009). While it is known that the composition of an aged dermis differs from young tissue, much is still unknown regarding how they differ. To gain a better understanding of what differences exist between young and old skin, we conducted a high throughput qPCR experiment to measure the activity of 72 genes over time in neonatal, adult and

irradiation-induced senescent fibroblasts. Since TGF- β is a key mediator positively regulating ECM homeostasis, we treated fibroblasts with TGF- β or a negative control to observe how the different cell lines respond.

The PCA and the differential expression analysis conducted in this study represent two different means of extracting information from this dataset. The PCA provides a broad overview of the data and clearly shows the measured genes respond differently in the different cell lines (Figure 4). On the other hand, the differential expression analysis provides a detailed indication of which genes behave differently in the compared groups. Notably, of all the comparisons made, the group containing the most differentially expressed genes was the comparison between TGF- β treated adult and neonatal fibroblasts (Figures 2a and 10a). The group with the second largest number of differentially expressed genes was the comparison within TGF- β treated adult cell lines. Conversely, the group with the least number of differentially expressed genes was within the neonatal control groups (Figure 2b and ??). The latter two findings are intuitive results because it is known that heterogeneity increases with age (ref). It is however interesting to note that in all comparisons, the TGF- β treated groups displayed greater levels differential expression than the control groups (Figure 2 and ??).

In Figure 3, we have selected a subset of the measured genes for discussion. Our measurements for type 1 collagen agree with previous reports that type 1 collagen is reduced with age. [Varani et al. \(2006\)](#) found that type 1 procollagen levels were 3 times lower in aged (80+) compared to young (18-29) individuals (15ng/mm² compared to 5ng/mm² respectively). Moreover, data presented in [Quan et al. \(2010\)](#) agrees with this assessment. Figure 3a and ?? shows that our data agree with these reports that TGF- β strongly induces the production of collagen from fibroblasts and that the amount produced is reduced in age. It is interesting to note that the amount of COL1A1 produced is approximately double that of COL1A2, in line with the knowledge that the stoichiometry for type 1 collagen heterotrimers two COL1A1 to one COL1A2 chain (ref).

TGF- β induces proliferation of fibroblasts and their differentiation to myofibroblasts ([Liu et al., 2016](#); [Negmadijanov et al., 2015](#)). Normally fibroblasts are in a quiescent state, controlling the normal homeostasis of dermal tissues. Under physiological responses such as wound repair, fibroblasts undergo differentiation and change their phenotype to an 'active' myofibroblast state which display characteristics of smooth muscle cells. α -SMA is a marker for myofibroblasts ([Zanotti et al., 2010](#); [Evans et al., 2003](#)) which facilitates contraction of a wound ([Darby and Hewitson, 2007](#)). Figure 3c therefore indicates that TGF- β induces differentiation of fibroblasts to myofibroblasts. Assuming α -SMA is an accurate marker for myofibroblasts, Figure 3c shows that adult fibroblasts ability to differentiate is severely impaired compared to neonatal and damage-induced senescent fibroblasts. This implies that when older cells needs to produce large quantities of ECM they are unable to do so with the same vigour as young cells. Therefore, lack of differentiability may mechanistically be related to why wound healing takes longer in the elderly.

CTGF is an important regulator of fibrotic signalling pathways and is a downstream target of TGF- β ([Quan et al. \(2002\)](#); [Wahab et al. \(2005\)](#); [Ponticos et al. \(2009\)](#)). Evidence that blocking CTGF signalling reduces the amount of collagen produced by sclerotic fibroblasts emphasises the relationship between CTGF and collagen levels ([Makino et al., 2017](#); [Sonnylal et al., 2010](#)). Our data agrees with previous reports that TGF- β strongly induces CTGF expression in fibroblasts Figure 3d. Moreover, our data agrees with ([Quan et al., 2010](#)) in that CTGF production is diminished in older fibroblasts.

Reduced collagen levels with age may result from both reduced production and increased degradation. MMPs are proteases in the extracellular matrix, some of which, including MMP1, can degrade collagen. It has been shown that the aged dermis produces higher levels of MMPs compared to younger individuals (ref). Specifically, ([Qin et al., 2017](#)) showed that the aged dermis contained higher levels of MMP1, MMP3, MMP9, MMP10, MMP11, MMP23, MMP24, MMP27 and MMP28 compared to young dermis. In agreement with [Qin et al. \(2017\)](#), our data suggest that Figure 3e MMP1 expression is enhanced in aged tissue compared to young and that a degree of heterogeneity exists

regarding MMP1 levels in age.

The MMP1 response to TGF- β (Figure 3e) is a surprising result that distinguishes this experiment from other reported studies. White et al. (2000) characterised a TGF- β inhibitory element (TIE) in the MMP1 promoter that is involved in constitutive MMP1 repression. Further, both White et al. (2000) and Edwards et al. (1996) observed that TGF- β inhibited PMA-induced MMP1 production, while (Yuan and Varga, 2001) observed that TGF- β inhibited IL-1 β production. These data point towards TGF- β mediated transcriptional repression of MMP1, which is an attractive idea because it is intuitive from a resource allocation point of view that if TGF- β directs anabolic processes, than catabolic processes should be inhibited. However, contrary to this hypothesis, MMP1 did not positively or negatively respond to TGF- β stimulation in any of the age or senescent cell lines and this result robust across all 9 cell lines under study (Figure 3e). A hypothesis to explain this apparent discrepancy is that in (White et al., 2000; Edwards et al., 1996; Yuan and Varga, 2001), TGF- β only inhibited an increase in MMP1 production that was induced by either TPA or IL-1 β . On the other hand, we have measured the MMP1 response to TGF- β or a negative control, without prior stimulation with a positive regulator of MMP1. Thus a plausible conclusion consistent with all the evidence is that TGF- β inhibits induced-MMP1 synthesis, but not basal MMP1 production. The time matched controls were pivotal in reaching this conclusion because if only a 0 time point control was used then it would appear as though TGF- β induced a reduction in MMP1 transcription. The data in Figure 3e emphasises the importance of time matched controls when studying the dynamics of biological systems.

Smad7 is the most important negative feedback of the Smad system (Hayashi et al., 1997; Nakao et al., 1997; Yan et al., 2016; von Gersdorff et al., 2000; Ebisawa et al., 2001; Hanyu et al., 2001; Pulaski et al., 2001; Suzuki et al., 2002; Shi et al., 2004; Zhang et al., 2007). Smad7 both negatively regulates Smad signalling and represents a mechanism of cross-talk with other signalling pathways (Yan and Chen (2011)). A considerable portion of the work that studied Smad7 has been conducted on keratinocyte (HaCaT) cell lines and indicate that Smad7 levels peak at approximately 1h post-TGF- β stimulation (Denissova et al. (2000)). Figure 3f provides evidence that in fibroblasts, Smad7 mRNA levels peaks between 1 and 3h post-TGF- β stimulation. In neonatal and senescent cell lines, a second peak in Smad7 production was observed 48-72h post-TGF- β stimulation. In adult cell lines the magnitude of the first peak is markedly reduced compared to neonatal cell lines. It is not clear what the biological purpose of this second peak in Smad7 production is but given that Smad7 inhibits the Smad signalling pathway, its likely that canonical Smad signalling is not active at these later time points. It is also unclear what the biological implications of reduced Smad7 production in age has on the TGF- β biology. It may be that Smad7 production is lower in adult cells because Smad signalling is impaired and requires less inhibition.

Smad3 is a prototypical effector of TGF- β signalling and essential for the transcription of type 1 collagens Runyan et al. (2003). Purohit et al. (2016) observed reduced Smad3 levels in both age and senescent fibroblasts and showed using silencing RNA experiments that reduced Smad3 could account for the observed reduction in type I procollagen in the aged dermis. While it is certainly feasible that less Smad3 in adult cells would cause reduced collagen production, Figure 3g only provides weak support for this hypothesis since the differences between adult and neonatal cell lines are not so profound. However, just because this data does not provide good support for a transcriptional mechanism of Smad3 decline in age does not preclude the possibility that Smad3 protein levels are reduced in age because of an alternative mechanism, such as enhanced Smad3 degradation. Therefore the role of Smad3 in ageing fibroblasts is still an open question, but based on this data it is unlikely to involve a transcriptional mechanism.

An interesting aspect of the Smad3 data is that 8-12h post stimulation by TGF- β , Smad3 mRNA levels are reduced to what appears to be a new steady state (Figure 3g). This observation suggests the existence of a late acting negative feedback in the TGF- β response to persistent TGF- β stimulation. To our knowledge, this insight into Smad signalling is novel. It is noteworthy that the timing of the drop in Smad3 mRNA levels directly precedes the incline in α -SMA

and so a hypothesis is that this drop in Smad3 mRNA occurs before or during fibroblast differentiation, though this would need experimental testing.

A limitation of this study is that we have measured only 72 genes. While it would have been more informative to measure all activity of all genes instead (for example by microarray or RNA-seq), the use of high throughput qPCR enabled the measurement of more experimental conditions. In turn, we were able to design our experiments as time series to measure the dynamics of gene expression over time. Despite choosing the 72 genes to be as relevant to the subject of skin ageing as possible, there are inevitably others which we have not been able to measure, and so our analysis is based on a bias selection of genes.

Biological function operates at the protein level of biological organisation, but we have only measured mRNA. While still valuable, since there is not necessarily a one-to-one correspondence between mRNA and protein ([Liu et al., 2016](#)), it would be illuminating to perform some parallel proteomic and phosphoproteomic experiments to provide a more comprehensive understanding of the differences between young and old fibroblast behaviour.

Another limitation of this work is that irradiation-induced senescence was used as a model for replicative senescence. It has been shown that there strong similarities between the replicative and irradiation-induced senescence ([Marthandan et al., 2016](#)), but there still may be important differences which should be considered when drawing conclusions about replicative senescence from a irradiation-induced senescence model.

In this work we have studied the dynamic response of three groups of cells: neonatal, adult and irradiation-induced senescent fibroblasts. We have shown that considerable differences exist between the response of these three cell types both in response to TGF- β and without stimulation. We have discussed a selection of the data and have built an interface which is available for interactively exploring both the time series and the PCA data. We envision that the data presented here combined with some protein level data will be useful for incorporation into mechanistic models that describing the differences in signal transduction biochemistry between young and old fibroblasts.

3 | MATERIALS AND METHODS

3.1 | Cell Culture

Three independent human neonatal dermal fibroblasts (HDFn) labelled A (Caucasian male, catalogue number: C-004-5C, lot number: #1366434), B (Caucasian male, catalogue number: C-004-5C, lot number: #1366356) and C (Caucasian male, catalogue number: C-004-5C, lot number: #1206197); three irradiation-induced senescent cell lines (D, E and, F) which are the same as A, B and C respectively but irradiated with 20Gy ten days prior to seeding, and three adult cell lines G (55 years old Caucasian female, catalogue number: C-013-5C, lot number: #1528526), H (60 year old, Caucasian male, catalogue number: A11634, lot number: #1090465) and I (65 year old, Caucasian female, catalogue number: A11636, lot number: #200910-901) were purchased from Life Technologies and seeded into standard tissue culture treated 12-well dishes at a density of 10,000 cells/cm² in 3.5ml M106 medium (ThermoFisher Scientific, catalogue number: M106500) supplemented with LSSG (ThermoFisher Scientific, catalogue number: S00310) and at 27°C, 5%CO₂ for 4 days. Senescent cells were seeded at higher density of 65,000 cells/cm².

3.2 | Treatment Protocol

Cells from each cell line were serum starved 24h prior to treatment by removing LSGS supplementation from media. Following 24h of incubation at 37°C and 5% CO₂, cells were assigned one of three treatments: baseline, control or TGF- β . Baseline samples were not treated in any way prior to harvesting at experiment start (0h) and end (96h).

TGF- β and control samples were treated with media containing 5ng mL $^{-1}$ TGF- β reconstituted in 10mM citric acid or 0.1% BSA in 10mM citric acid respectively. In control and TGF- β groups, cells were harvested at 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96 hours post treatment. All 216 conditions were repeated 6 times resulting in 1296 individual samples. Samples were shipped to Procter and Gamble (P&G), Cincinnati for quantification on a high throughput PCR Smart chip platform by WaferGen.

3.3 | High throughput qPCR smart chip

-include the randomisation procedure used on the plates.

3.4 | Normalization

The raw data (containing cycle threshold values) is available for download in supplementary file 2. Each gene measured in each sample was normalised to PPIA, the gene which encodes for peptidylprolyl isomerase A which is stationary throughout treatment with TGF- β . Equation (1) was used to normalise every gene g of every sample prior to visualisation and differential expression calculations.

$$\Delta CT_g = 2^{-CT_g - CT_{PPIA}} \quad (1)$$

The normalised data is available for download from the associated website that hosts the data visualisation application.

3.5 | Differential Expression

The R package, LIMMA ([Smyth, 2004, 2005; Ritchie et al., 2015](#)), was used to compute differential expression statistics. LIMMA was configured to using a spline matrix with 4 degrees of freedom, as per the LIMMA user guide, to compare either the control time series between adult/senescent and neonatal cell lines or the TGF- β treated cells. To make use of the available cell line replicates, differential expression calculation was repeated so that all adult/senescent cell lines were compared with all neonatal cell lines. The results were aggregated by calculating the percentage of times a gene has a false discovery rate (FDR) corrected p-value smaller than 0.05. This was repeated for both control and TGF- β data sets and for both adult and senescent cell lines, compared with neonatal. The R script used is available for download in supplementary file 3.

3.6 | Website Development

The website was developed using the Django (version 2.0.5) web framework using Python 3.6. Plotting was conducted using Bokeh and Plotly, which are both Python visualisation libraries. The source code used to build the website is available at https://github.com/CiaranWelsh/data_viz_with_django.

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support information.

conflict of interest

You may be asked to provide a conflict of interest statement during the submission process. Please check the journal's author guidelines for details on what to include in this section. Please ensure you liaise with all co-authors to confirm agreement with the final statement.

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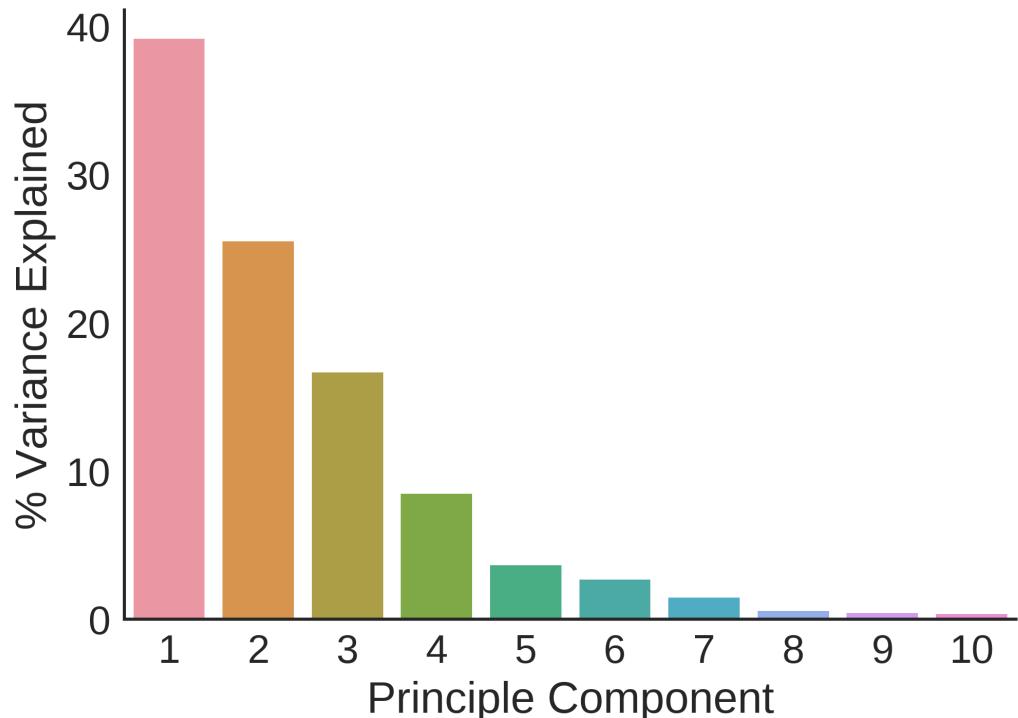


FIGURE 5 Plot showing the percent of variance explained for the first 10 principal components.

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4 | SUPPLEMENTARY FIGURES

4.1 | Principle Component Analysis

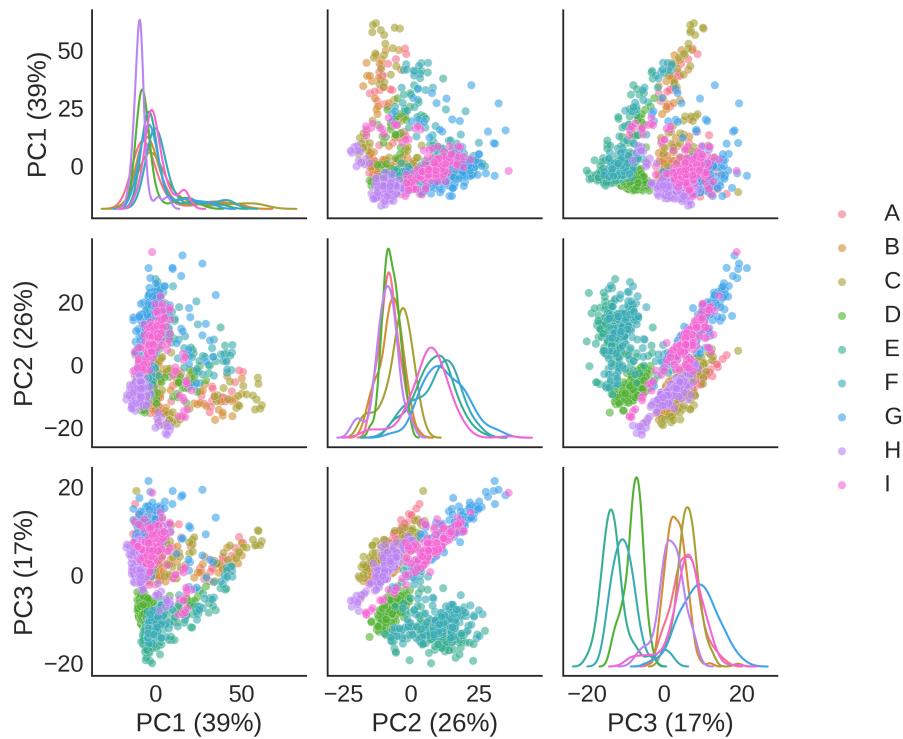


FIGURE 6 Scatter matrix of first three principle components coloured by cell id.

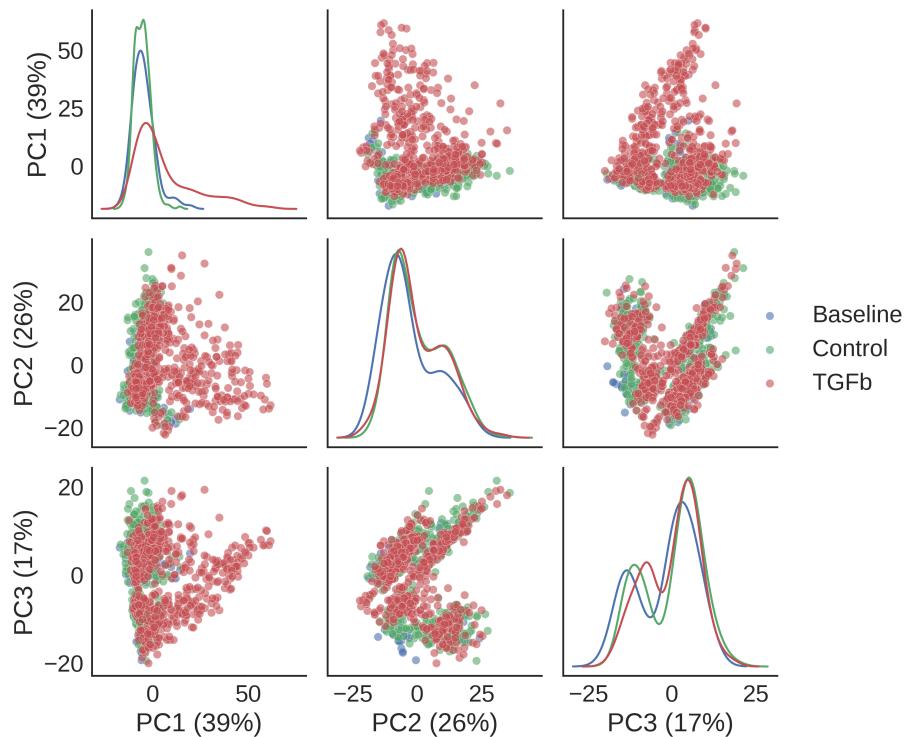


FIGURE 7 Scatter matrix of first three principle components coloured by treatment.

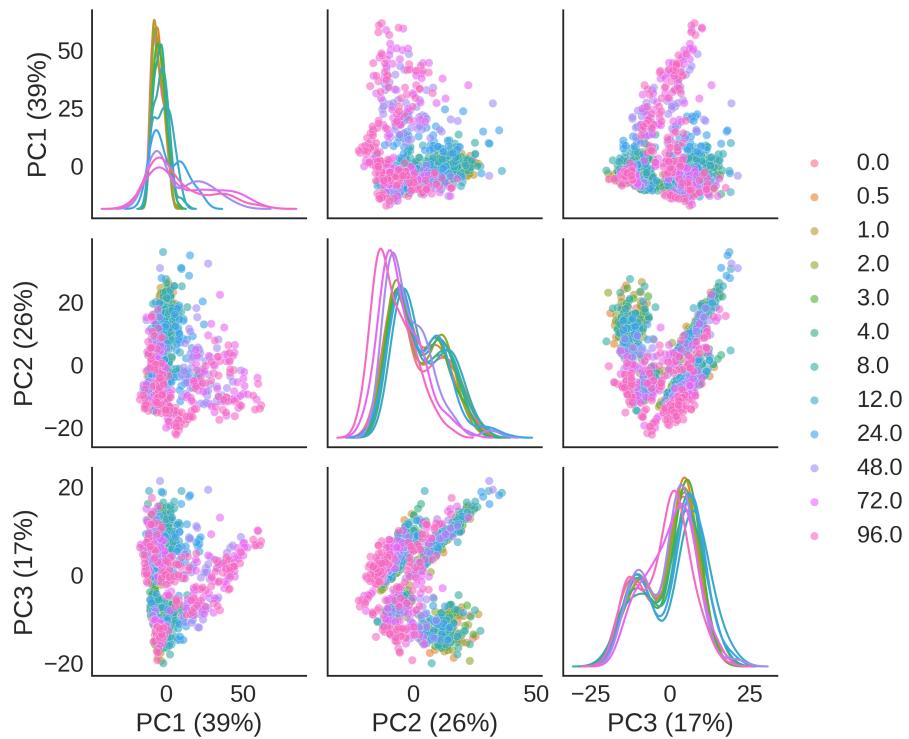


FIGURE 8 Scatter matrix of first three principle components coloured by time point.

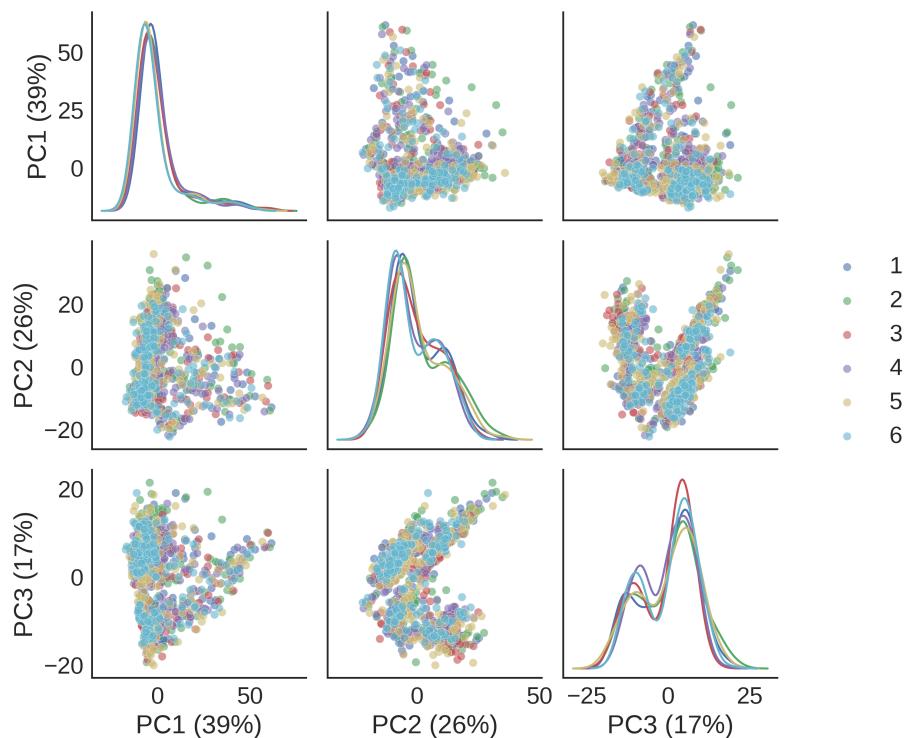


FIGURE 9 Scatter matrix of first three principle components coloured by replicate.

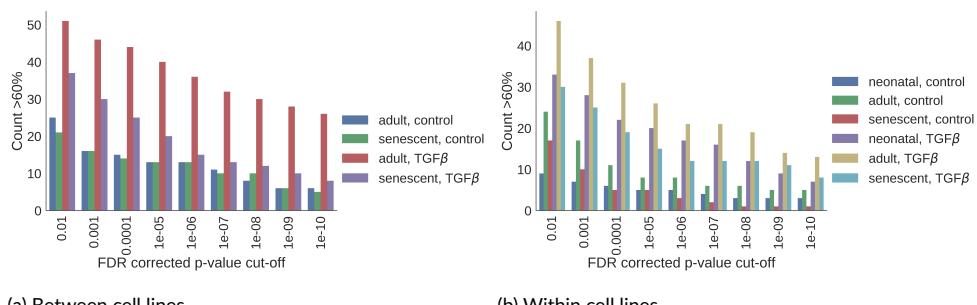


FIGURE 10 Counts of genes that were differentially expressed in >60% of LIMMA analyses as a function of p-value for (a) the between groups and (b) within groups comparisons.