Polymorphic dynamics of ribosomal proteins gene expression during somatic cell reprogramming and their differentiation in to specialized cells-types

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Abbreviations: DBA: Diamond Blackfan Anaemia; ESCs: Embryonic Stem Cells; HDF: Human dermal fibroblasts; HA: Human astrocytes; iPSCS: induced pluripotent stem cells (iPSCs); NHBE: Normal human bronchial epithelium; PCR: Polymerase chain reaction; prEC: Human prostate epithelial cell; OSKM: OCT4, SOX2, KLF4 and c-MYC; RP: Ribosomal Protein;

Abstract:

Factor induced pluripotent stem cells (iPSCs) offer great promise in regenerative medicine. However, accumulating evidence suggests that iPSCs are heterogeneous in comparison with embryonic stem cells (ESCs), and that is attributed to various genetic and epigenetic states of donor cells. In the light of the discovery of cell-type specialized ribosomal protein composition, its role as the cells transit through different stages of reprogramming and when iPSCs differentiate into specialized cell-types has not been explored to understand its influence in the reprogramming and differentiation process and outcome. By re-analyzing the publicly available gene expression datasets among ESCs, various sources of iPSCs and somatic cells and by studying the ribosomal protein gene expression during different stages of reprogramming of somatic cells and different passages of established iPSCs we found distinct patterns of their expression across multiple cell-types. We experimentally validated these results on the cells undergoing reprogramming from human dermal fibroblasts. Finally, by comparing publicly available data from iPSCs, iPSCs derived specialized cells and it's *in vivo* counterparts, we show alterations in ribosomal gene expression during differentiation of specialized cells from iPSCs which may have Implications in the context of ribosomopathies. Our results provide an informatics framework for researchers in efficient generation of iPSCs that are equivalent to ESCs.

Introduction

Pluripotent stem cells (PSCs) have tremendous applications in developmental studies, disease modelling and regenerative therapy [1]. Induced pluripotent stem cells (iPSCs), generated from adult somatic cells by ectopic expression of reprogramming factors, possess properties similar to embryonic stem cells (ESCs), and have been explored as a potential replacement for ESCs in downstream applications [2]. Initially, iPSCs were thought to be very similar to ESCs but later they were found to be substantially different in their gene expression patterns [3]. Irrespective of the source of their donor cell-type, iPSCs were shown to be less efficient in their differentiation potency to other cell-types but were shown to easily differentiate into their respective donor cell-type, highlighting the influence of donor cell-type specific epigenetic memory in this process [4]. However it was noted that continuous passaging of these cells would attenuate these differences between iPSCs of different sources [5]. Like donor cell-lineage specific factors, incomplete DNA methylation, incomplete repression and reactivation of multiple genes [6], persistent donor cell-type specific gene expression or unique gene expression pattern [7] have been attributed to these observed phenomena. The transcriptional/post-transcriptional regulation of these aberrant/unique epigenetic signatures of iPSCs is poorly understood but errors arising during reprogramming or incomplete reversion to pluripotency could be a cause. Since the potential application of iPSCs in regenerative medicine and disease modelling depends on successful cell-type specific differentiation of iPSC, one needs to investigate mechanisms behind reprogramming and differentiation.

In addition to the above mentioned epigenetic determinants and other regulatory components of transcription [2, 8, 9] and post-transcription [10, 11], the components of translation might also influence restricted differentiation of iPSCs. Multiple studies conducted in cell-types ranging from bacteria to malignant cells indicate the existence of ribosomal subpopulations that differ in their protein complement cause diverse functional translational machinery [12-14]. In this regard the occurrence of cell-type specific ribosome composition particularly during generation of iPSCs has attracted our attention. Researchers have reported that ribosome composition is tissue specific and expression levels of different ribosomal proteins (RPs) are different in different tissues/cell-types [15-17]. Interestingly decrease in concentration of a specific RP was shown to affect a spectrum of translated mRNAs without

affecting overall protein synthesis in a given cell [18]. This explanation could account for the fact that mutations in some of the RP genes cause abnormality in particular tissue or cell-type, but doesn't affect the whole body of an organism [19]. Recent mass spectrometric studies on RPs among different cell-types reported by Slavov *et al*., [20] further support the existence of ribosomes with distinct protein compositions and physiological functions. The recent study [21] reveals a more concrete functional link between heterogeneity in ribosome composition and translational circuitry in mouse ESC. Based on these observations, we hypothesized that heterogeneity in cell-type specific ribosome composition could serve as one of the important determinants that might restrict iPSCs to achieve complete pluripotency.

Here, we first analyzed expression pattern of RP genes during different days of reprogramming of four somatic cells to respective iPSCs and compared them with that of human ESC and report distinct patterns in the RP gene expression. Later, we observed these patterns persist in established iPSC lines at extended passages. Finally, we analyzed expression profiles of iPSCs derived specialized cells and their *in vivo* counterparts. Our analysis identified the unusual polymorphic behaviour of various RP gene expressions during this process. These results highlight the importance of ribosome composition in reprogramming of somatic cells and differentiation of iPSCs to specialized cell-types.

Material and Methods:

Bioinformatics analysis of expression patterns during somatic cell reprograming:

The publicly available microarray gene expression data sets (GSE50206) of human ESCs and four different somatic cell-types viz. human dermal fibroblasts (HDF), human astrocytes (HA), normal human bronchial epithelium (NHBE) and human prostate epithelial cell (prEC) that were subjected to reprogramming were analysed [22]. The 75th percentile normalized expression values were downloaded for analysis of RP gene expression. We divided each dataset that consists of ESCs, donor cell and iPSCs derived from a particular donor cell-type into two parts. One with an expression range of -0.5 to +0.5 (range1) and the rest in another part (range2). We extended this to all other cell-types. Next we designed a practical extraction and report language (PERL) program to identify expression state of a given gene. If the gene is expressed the expression level value will be greater than 0.3 will be in range1 and 0.4 in range2 and if the gene is not expressed or down-regulated the value, which is less than -0.2 in range 1 and if it is less than -0.3 in range 2. The thresholds we selected because at these values, the eight expression patterns that were described in this study could be clearly seen under a heat map. Lesser values were not considered as they may hinder the significance of these results. We set minimum threshold to consider a value as “not expressed”/”very less expressed” and an upper threshold to consider as “overexpressed”. We set the parameters for each of the nine expression patterns and applied for this program with same thresholds for all the cell types. The heatmap representing ribosomal genes’ expression (Fig2) was drawn using Java treeview software tool [23].

Derivation of iPSC lines and fluorescence activated cell sorting (FACS) of reprogramming cells:

Human adult dermal fibroblast was subjected to reprogramming using STEMCAA lentiviral vector using Bharathan et al., (2017) protocol [24]. On day 12 of reprogramming, a single cell suspension was prepared by treating the cells with TrypLE (Gibco). The cells were stained with labeled antibodies, CD13-PE, SSEA-4-Alexaflour647 and TRA-1-60-BV421 (BD Pharmigen) in KOSR based human iPSC medium for 30 minutes at 4^{0}C in dark. The stained cells were washed twice with 1X PBS and sorted using FACS Aria III flow cytometer. Based on the co-expression pattern of three markers CD13, SSEA-4 and TRA-1-60, the cells were sorted into four fractions,

$CD13^{+ve}$ $SSEA\text{-}4^{-ve}$ $TRA\text{-}1\text{-}60^{-ve}$, $CD13^{+ve}$ $SSEA\text{-}4^{+ve}$ $TRA\text{-}1\text{-}60^{-ve}$, $CD13^{-ve}$ $SSEA\text{-}4^{+ve}$ $TRA\text{-}1\text{-}60^{-ve}$ and $CD13^{-ve}$ $SSEA\text{-}4^{+ve}$ $TRA\text{-}1\text{-}60^{+ve}$. The sorted cells were centrifuged, cell pellet was re-suspended in Tri-reagent and stored at $-80^{0}C$.

Derivation and establishment different passages of HDF derived iPSCs:

The iPSC lines were derived from HDFs by overexpression of OCT4, SOX2, KLF4 and c-MYC (OSKM) using retroviral factor delivery method [2]. The colonies were isolated based on hESC-like morphology, maintained on SNL feeder layers in hiPSC medium and characterized for pluripotency [24]. The fully characterized and established hiPSC lines were maintained in extended cultures on SNL feeders in hiPSC medium and passaged using collagenase-IV treatment. iPSCs representing passage-5 (P-5), P-27, P-43, P-65, P-71 were collected, centrifuged, cell pellet was re-suspended in Tri-reagent and stored at $-80^{O}C$.

RNA isolation and quantitative PCR analysis:

RNA was extracted from fibroblasts, sorted reprogramming cells and iPSC lines using Tri-reagent (Sigma-Aldrich). 1 µg of total RNA was used for reverse transcription reaction using Primescript RT reagent kit (Takara) according to the manufacturer's instructions. Quantitative RT-PCR was set up with SYBR Premix Ex Taq II (Takara Bio) using specific RP gene primers (supplementary Table 1) and analyzed on QuantStudio12K Flex (Life Technologies) real-time PCR systems. The raw data was normalized with *ACTB* gene expression.

Bioinformatics analysis of RNA-seq data sets from iPSC derived adult cells:

RNA-Seq data from iPSCs, iPSCs derived specialized cells and their respective *in vivo* counterparts were downloaded from NCBI GEO with accession numbers (Supplementary Table 2) and the expression values were converted to log2 TPM (transcripts per million) [25]. Then the expression values of RP genes and the house keeping genes *ACTB* and *GAPDH* were taken and plotted on heatmap using R.

Results:

Dynamic expression of RP gene expression during somatic cell reprogramming:

Analysis of publicly available microarray gene expression profiles of pluripotent ESCs, different days of reprogramming donor cells-types of NHEB, HDF, HA and PrEC revealed distinct patterns of RP gene expression (Fig 1a (NHEB cells); 1b (HDF cells); 1c (HA cells) & 1d (prEC cells). The time frame of reprogramming process was divided into early, intermediate and late days to define the transitions in expression profile. During this time frame the RP genes were identified to exhibit eight patterns of expression among these four cell-types (Fig 2). In pattern 1, the gene expression levels of reprogramming cells in early days were similar to donor somatic cells and that in late days were similar to that of ESCs (Fig 2a). This indicated that the genes belonging to this category show donor specific expression in the early days and reprogramming factors could easily bring about the shift in expression profile during reprogramming. Gene with pattern 2 retained donor cell-type specific transcriptional profile in early and late days of reprogramming (Fig 2b). The persistent expression pattern of these genes may contribute to donor cell memory in reprogramming cells. Genes exhibiting pattern 3 showed a higher level expression in intermediate days, followed by attaining expression level similar to donor cell-type in late days (Fig 2c). Genes with pattern 4 showed a higher level expression in intermediate days, followed by attaining expression level similar to ESCs in late days (Fig2 d). Genes with pattern 5 exhibited expression level varying from high level in donor cells and reprogramming cells in early days, intermediate level in ESCs and low level in reprogramming cells in late days (Fig 2e). In Pattern 6, gene expression was observed only in the intermediate days of reprogramming and not in ESCs, donor cells or reprogramming cells in later days (Fig 2f). Genes exhibiting pattern 7 were expressed only in reprogramming cells and are not expressed in donor nor in ESC (Fig 2g). Genes with pattern 8 showed expression at the low level in ESCs, intermediate level in donor cells and high in reprogramming cells in late days (Fig 2h). However, it has to be mentioned that expression of a given ribosome protein is not only dynamic but follow different patterns of expression among different cell-types. Overall, most of the genes belonged to Pattern 1 (Fig 2a) wherein the donor cell memory is retained in immediate stages but gets erased over the time and show ESCs type expression at late stage passages. All the genes belonging to various categories in different cell-types described above are listed (Figure 3a).

Validation of *in silico* observed RP gene expression during somatic cell reprograming.

For the validation of *in silico* data on dynamic expression of RP genes, we took the advantage of recently established method of isolation of various stages of OSKM induced reprogramming of HDF by fluorescence activated cell sorting [24] (see methods). The reprogramming cells were sorted based on expression of fibroblast marker, CD13 and pluripotency markers SSEA-4 and TRA-1-60 to obtain cells belonging to different stages of reprogramming namely, fibroblast stage ($CD13^{+ve}$ $SSEA4^{-ve}$ $TRA\text{-}160^{-ve)}$, intermediate stages ($CD13^{+ve}$ $SSEA4^{+ve}$ $TRA\text{-}160^{-ve}$ and $CD13^{-ve}$ $SSEA\text{-}4^{+ve}$ $TRA\text{-}160^{-ve}$), and late stage ($CD13^{-ve}$ $SSEA\text{-}4^{+ve}$ $TRA\text{-}160^{+ve}$) (22), and were compared with control iPSC lines, CR5 and BC1 by quantitative PCR. The fourteen RP genes showed varying expression levels in fibroblasts, reprogramming cells at different stages and control iPSC lines (Fig 3a). The fibroblasts showed least level of expression for all analysed RP genes and when subjected to reprogramming, an increase their expression levels were observed, which peaked at the intermediate stage, $CD13^{-ve}SSEA\text{-}4^{+ve}TRA\text{-}1\text{-}60^{-ve}$. This expression pattern was prominent with *RPL17* and *RPS29*. It was observed that the reprogramming cells at the late stage, $CD13^{-ve}$ $SSEA\text{-}4^{+ve}$ $TRA\text{-}1\text{-}60^{+ve}$ expressed the genes at levels higher than the control iPSC lines. This pattern is clearly evident for *RPL23A*, *RPS9*, *RPS18* and RPS29.

Dynamic expression of RP genes at various passages of established iPSCs:

To check whether these expression patterns continue even after the establishment of induced pluripotency, we analysed established iPSC lines at early (P-27), intermediate (P-43) and late passages (P-65 & 71). The RP gene expression pattern was estimated by quantitative PCR. Interestingly, we observed that most of the genes show similar dynamics during extended culture of iPSC lines and genes like *RPL15*, *RPL17*, *RPL28, RPL37*, *RPS6*, *RPS9* and *RPS18* showed higher expressions at later passages (Fig 3b). Strikingly, these genes show decreased expression from P-43 to P-65 and then gradually show elevated expression in subsequent passage stages P-71 (Fig 3b).

Dynamic expression of RP gene expression in specialized cells derived from established iPSCs

Differentiation of iPSCs to specialized cell-types is one of the major research focuses in developing iPSCs based regenerative therapy. The RP gene expression patterns in the late passages of the iPSC might influence their differentiation and thereby have an impact on the properties of derived specialized cells. To investigate this possibility, we analysed publicly available RNA seq data for expression patterns of the ribosomal genes in multiple sources of iPSCs derived specialized cells such as neurons and CD34+ hematopoietic stem cells (Supplementary Table 2 & 3). Indeed, we found that the genes such as *RPL7*, *RPL17*, *RPL23A*, *RPS7*, *RPS10* and *RPS27* showed significantly lower expression levels in iPSCs derived specialized neurons when compared with its parental iPSCs and adult neurons (Fig 4a). Other genes such as *RPL28*, *RPL37* and *RPS18* showed similar patterns with lower variations in expression levels [Fig 4a]. These genes were categorized under pattern 8, which were hypothesized to continue their higher expression in the differentiated cells, neurons in this case. More interestingly, genes such as *RPL9*, *RPL10*, *RPL14*, *RPL24*, *RPL34*, *RPL39*, and *RPS19*, which were not categorized into pattern 8, show a dramatic drop in the gene expression levels compared to iPSC and adult neurons. [Fig 4b] When a similar comparison was made between adult CD34+ hematopoietic stem cells and iPSC (reprogrammed from bone marrow cells) derived CD34+ cells, many genes such as *RPL10*, *RPL13*, *RPL15*, *RPL17*, *RPL21* (Fig4c) showed increased expression in iPSC derived CD4+ cells than their *in vivo* counterpart [Fig 4c] *RPS6KA3,* a protein kinase of *RPS6*, was observed to be deficient in all the somatic cells at late stages of reprogramming. But in the iPSC derived neural cells, there seems to be slightly higher expression than that of neurons.

Discussion:

The observations described in this study in the context of reprogramming of somatic cells and differentiation of iPSCs highlight the importance of RP genes in pluripotency. The RP genes were found to show the distinct pattern of expression during the course of somatic cell reprogramming (Table 1). Hence, regulating the expression of these genes during pluripotency induction may potentially influence the outcome of reprogramming. Abnormal expression of RPs in iPSCs may influence the features of cells differentiated from them and thereby can result in disease phenotypes. The patterns we described in cells during somatic cell reprogramming provide a comprehensive and polymorphic dynamics of RPs gene expression during this process. The RP genes with patterns such as 8 and 9 could be

manipulated so that iPSCs will attain expression pattern similar to ESCs. Similarly, further studies on RP genes showing pattern-7 which are expressed highly only in iPSCs, may aid in a better understanding of the process of factor induced reprogramming. Strikingly, these patterns persist in established iPSCs that are maintained in culture for many passages. These patterns give information about the polymorphic behaviour of RP genes dynamic expression in reprogramming cells from four different somatic donor cell-types, which may be helpful in choosing the appropriate donor cell-type for reprogramming and then differentiating them into specialized cell-types.

In the protocol which was used to reprogram HDF to iPSC by Takahashi et al [22], they have considered the reprograming up to 49 days from the day of induction and the later days of reprogramming were considered around day-42 and day-49. However, in the protocol by which we generated iPSC from HDF, the later stage of reprogramming is around day-20. This might possibly the reason why our qPCR data during reprogramming is not accurate in accordance with the data from Takahashi et al., [2]. Considering the fact that established iPSCs were reprogrammed only for 20 days in our protocol, these patterns might be again due to the fact that the early passages from established iPSC might be equivalent to the late passages of Takahashi et al., [2]. Despite that, many genes show pattern-8, i.e., elevated expression in later iPSCs, even though the cells were passaged up to 71 times (Fig 3b).

Analysis of specialized cells such as neurons and CD34+ hematopoietic cells derived from iPSCs with their *in vivo* counterparts, the expression patterns of some of the RP genes were found to be different. The patterns in neural cells differentiated from HDF derived iPSCs are different from CD34+ cells differentiated from bone marrow derived iPSCs. The differences we observed here can be partly attributed to the differences in protocols used for factor induced reprogramming and iPSC differentiation or due to inherent cell-type specific genetic and epigenetic differences. In this regard, deficiency of certain RPs in iPSCs derived neurons and CD34+ cells may lead to ribosomopathies. For example, deficiency of *Rpl17* in mouse resulted in enhanced production of shortened 5.8S rRNA [26] Similarly mutations in *Rps7* in mouse is associated with Diamond-Blackfan anaemia (DBA) and neuroanatomical phenotypes [27]and mutations in *RPS19* in humans with DBA [28].

Based on dynamic expression patterns of various RP genes during factor mediated somatic cell reprogramming and at different passages of established iPSCs, it would be predictable that perhaps knocking down selective factors from iPSCs would pave their differentiation towards a specialized cells-types so as to enable them to express similar levels of RP genes as that of its *in vivo* counterparts. However, RP gene expression analysis in specialized cells derived from iPSCs were found to be quite different from both of its *in vivo* counter parts and parental iPSCs themselves, suggesting that heterogeneity in RP genes expression could arise during somatic cell reprogramming, and also during iPSCs differentiation to specialized cell types, reinforcing the fact that one need to carefully evaluate and manipulate their expression profiles before using them for regenerative therapy.

Here in our study, we emphasize the importance of considering the heterogeneity in ribosome composition among various iPSC lines as it can influence their differentiation potential. This study provides a clue that RP composition play an important role in cell-type specific gene regulation and highlights the role of specialized ribosomes in determining the properties of iPSCs. Further elaborate studies need to be conducted to understand the mechanisms of pluripotency and differentiation process of iPSCs for their application in regenerative medicine.

Conclusions:

First, we observed and derived dynamics of Ribosomal proteins' gene expression during factor induced reprogramming from published datasets. Most of the RP genes in iPSC show similar expression as in that of mESC. Some genes, like in pattern 8, are to be considered for manipulation to obtain expression similar to that of ESC, to avoid the persistent expression in adult cells derived from these iPSC, which may lead to Ribosomopathies. Some of these patterns continued in several passages of iPSC culturing after the establishment of iPSC state. Strikingly, when the expression data from iPSC derived adult cells were observed, many RP genes' expression is very different from their iPSC as well as from their *in vivo* counterparts. This suggests the need for further studies during generation and differentiation of iPSCs

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Figure legends

Figure 1: Dynamic expression of RP genes during somatic cell reprogramming: Heat map of group of RP genes showing spectrum of differential gene expression patterns at different days of reprogramming of HDF (**1a**), NHEB cells (**1b**), HAs (**1c**), prEC (**1d**) in comparison with ESCs and respective donor cell-types. Scale bar showing intensity scaled log2 RMA values corresponds to level of expression. Red, black and green colours indicate high, intermediate and low levels of expression respectively. HDF: human dermal fibroblasts (HDF), NHBC: normal human bronchial **e**pithelial cells, HA: human astrocytes, prEC: prostate epithelial cells, ESCs: embryonic stem cells.

Figure 2: **Dynamic patterns (1-8) of RP genes expression during somatic cell reprogramming**: Line graphs showing representative RP genes showing pattern 1-8 at different days of either NHEB, HA, prEC somatic cell reprogramming, in comparison with ESCs and respective donor cell-types (2a, b, c, d, e, f, g & h). In patterns-1, expression levels similar to donor cell in early days of reprogramming followed by attaining levels similar to ESCs in late days (**2a**). Pattern-2, expression levels in early and late days, similar to donor cells (**2b**), pattern-3, show higher level expression in intermediate days, followed by attaining expression level similar to donor cell-type in late days (**2c**), pattern-4, show higher level expression in intermediate days, followed by attaining expression level similar to ESCs in later days (**2d**), pattern-5, show higher levels in donor cells and levels decrease in early, intermediate days which are similar to that of ESCs and levels goes further down in later days (**2e**), pattern-6, show higher expression levels only in in the intermediate days of reprogramming but not in ESCs, donor cells or later days of reprogramming cells (**2f**), pattern-7, show expression only in early, intermediate and late days of reprogramming cells but not in donor cell or ESCs (**2g**) and pattern-8, show low levels of expression in ESCs and donor cells but steadily increase in intermediate and late days of reprogramming cells (**2h**) NHBE: normal human bronchial epithelial cells, HA: human astrocytes, PrEC: prostate epithelial cells. ESC: embryonic stem cells. RP genes following representative pattern are depicted in each graph.

Figure 3: **Quantitative PCR validation of RP gene expression during HDF reprogramming and at different passages of established HDF derived iPSCs**: (**3a**) Real-time PCR validation of selected RP gene expression during various days reprogramming of HDF in comparison with

HDF and established iPSCs (CR5 & BC1) showing peak expression in intermediate stages of reprogramming. (**3b**) Real-time PCR validation of selected RP genes in established hiPSC lines derived from HDFs at different passages (P-5, P-27, P-43, P-65 and P-71), showing dynamic patterns of expression. Values are normalized to *ACTIN-B* and fibroblasts (P-5)- ddCt method (see methods for details).

Figure 4: **Dynamic expression of RP genes among native and iPSCs derived specialized cells**: (**4a**) RP genes which showed pattern-8 of expression during somatic cell reprogramming, showing lower levels of expression in iPSCs derived neural cells than that of its *in vivo* neurons. Expression levels of RP genes in human ESCs and human iPSCs were shown for comparison. (**4b**) RP genes which do not follow pattern-8 of expression during somatic cell reprogramming, showing much lower levels of expression in iPSCs derived neural cells than that of its *in vivo* neurons. Expression levels of RP genes in human ESCs and human iPSCs were shown for comparison. (**5c**) RP genes which show relatively higher levels of expression in iPSCs derived CD4+ve cells than their *in vivo* counterparts. Human ESCs and human iPSCs were shown for comparison.

Table1 : Summary of RP genes following various patterns of expression during somatic cell reprogramming of various donor cell-types: Table showing dynamic pattern of expression followed a specific RP genes either in HDF, NHEB, HA or prEC. Those highlighted in yellow show the typical expected expression pattern upon induction. Those highlighted in red and green were showing variable patterns of expression, which has to be knocked down or over expressed respectively, for efficient reprogramming.

Supplementary Table 1: List of RP gene specific primers used in quantitative PCR during different stages of reprogramming HDFs and different passages of established HDF derived iPSCs.

Supplementary Table 2: List of publicly available RNA-seq data sets from Gene expression omnibus (GEO) that were used in this study, their origin of cell-types and their respective GEO accession numbers.

Supplementary Table 3: List of all RP genes and their log2 transformed, TPM converted expression values derived from publicly available RNA-seq data sets from hESCs, hiPSCs (1-2), neuronal cells derived from HDF iPSC (i-neural 1-3), i*n vivo* neurons (1-2), CD4 cells derived from bone marrow iPSCs.

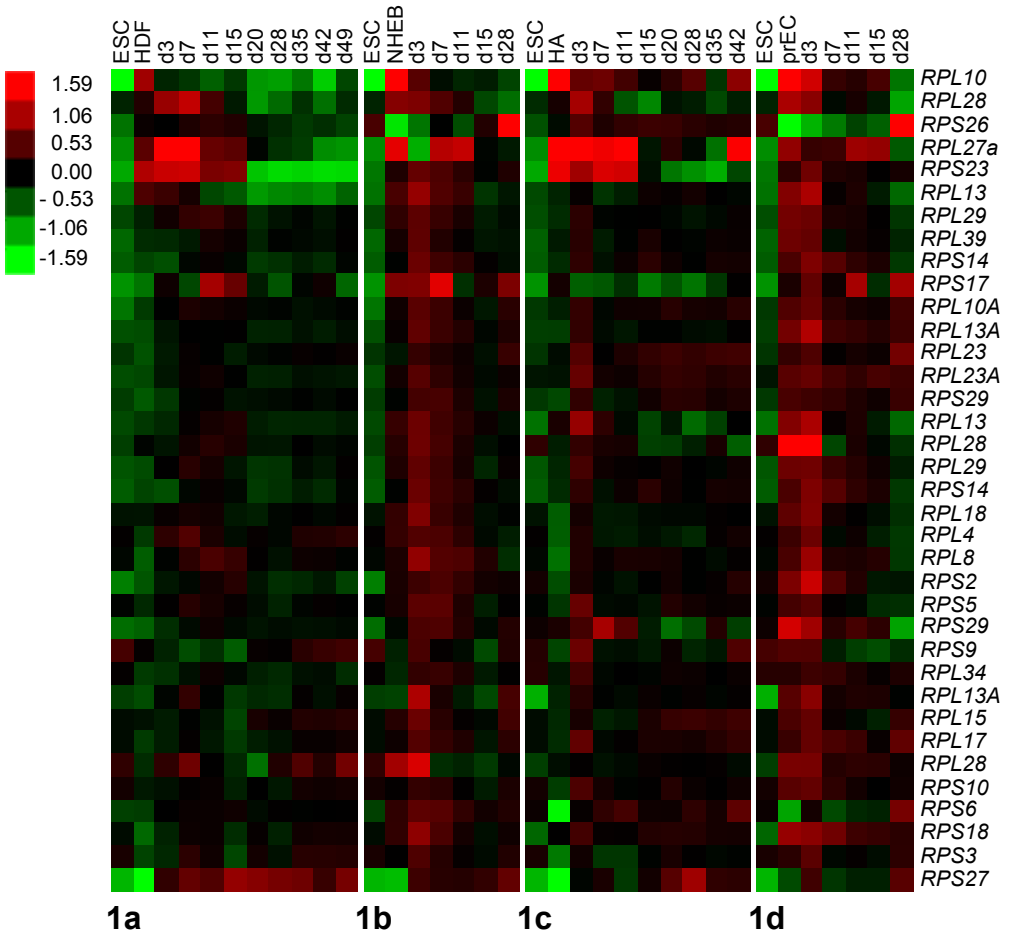


Figure 1: Dynamic expression of RP genes during somatic cell reprogramming: Heat map of group of RP genes showing spectrum of expression patterns during different days of reprogramming in HDF (**1a**), NHEB cells (**1b**), HAs (**1c**), prEC (**1d**) in comparison with ESCs and respective donor cell-types. Scale bar showing intensity of signal corresponds to level of expression. Red, black and green colours indicate high, intermediate and low levels of expression respectively. HDF: human dermal fibroblasts (HDF), NHBC: normal human bronchial epithelial cells, HA: human astrocytes, prEC: prostate epithelial cells, ESCs: embryonic stem cells

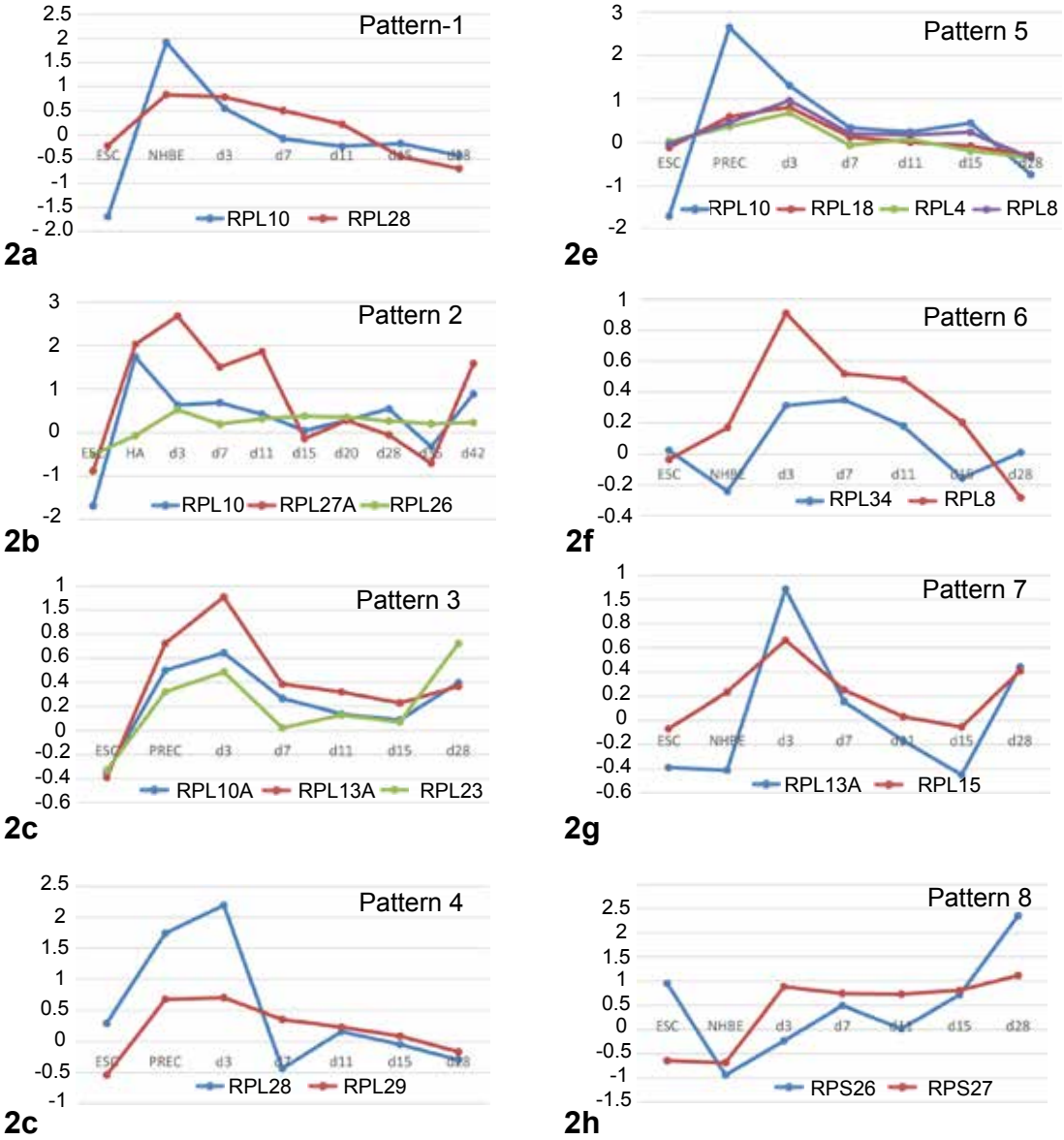


Figure 2: **Dynamic patterns (1-8) of RP genes expression during somatic cell reprogramming:** Line graphs showing representative RP genes showing pattern 1-8 at different days of either NHEB, HA, prEC somatic cell reprogramming, in comparison with ESCs and respective donor cell-types (2a, b, c, d, e, f, g & h). In patterns-1, expression levels similar to donor cell in early days of reprogramming followed by attaining levels similar to ESCs in late days (**2a**). Pattern-2, expression levels in early and late days, similar to donor cells (**2b**), pattern-3, show higher level expression in intermediate days, followed by attaining expression level similar to donor cell-type in late days (**2c**), pattern-4, show higher level expression in intermediate days, followed by attaining expression level similar to ESCs in later days (**2d**), pattern-5, show higher levels in donor cells and levels decrease in early, intermediate days which are similar to that of ESCs and levels goes further down in later days (**2e**), pattern-6, show higher expression levels only in in the intermediate days of reprogramming but not in ESCs, donor cells or later days of reprogramming cells (**2f**), pattern-7, show expression only in early, intermediate and late days of reprogramming cells but not in donor cell or ESCs (**2g**) and pattern-8, show low levels of expression in ESCs and donor cells but steadily increase in intermediate and late days of reprogramming cells (**2h**) NHBE: normal human bronchial epithelial cells, HA: human astrocytes, PrEC: prostate epithelial cells. ESC: embryonic stem cells. RP genes following representative pattern are depicted in each graph.

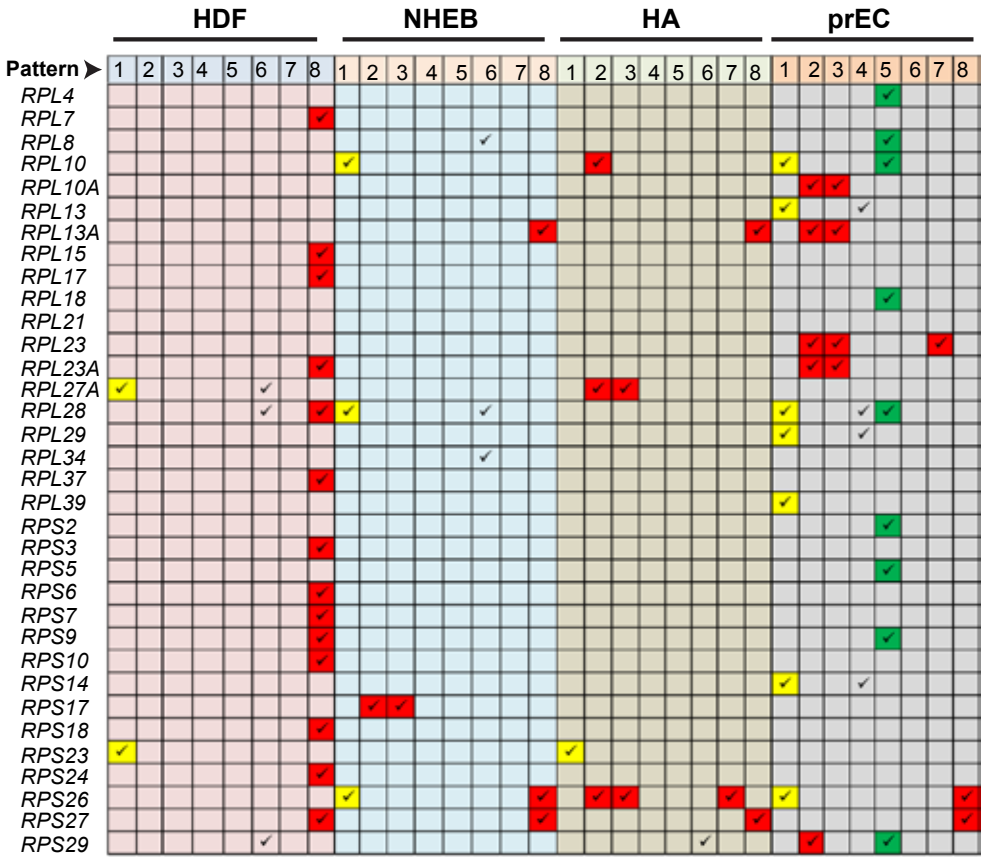
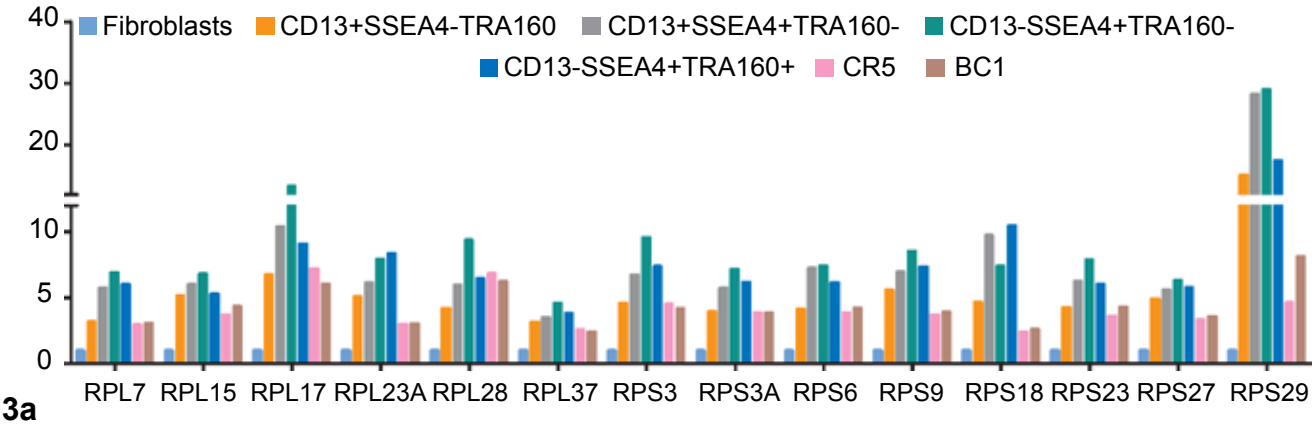
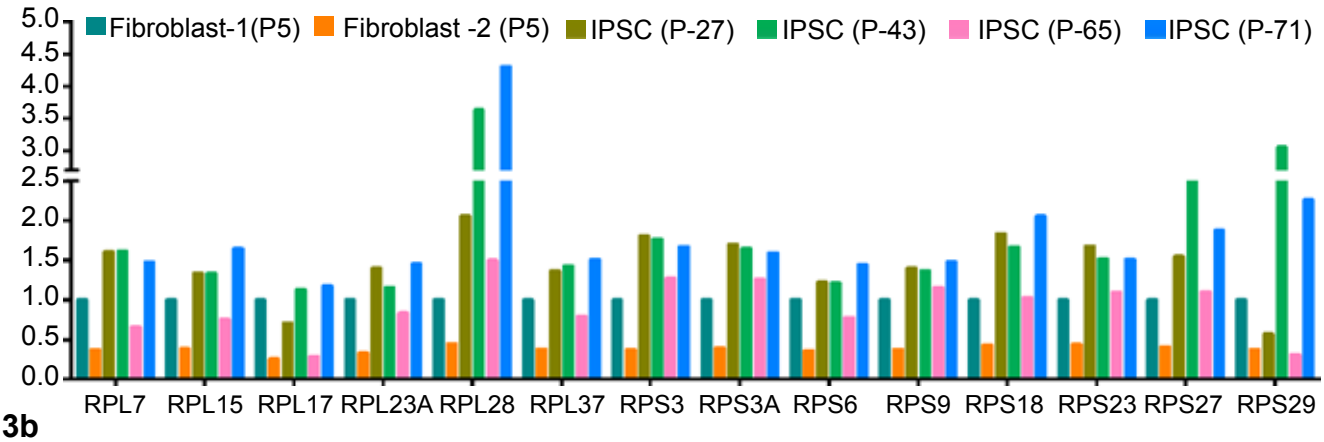


Table1 : Summary of RP genes following various patterns of expression during somatic cell reprogramming of various donor cell-types: Table showing dynamic pattern of expression followed a specific RP genes either in HDF, NHEB, HA or prEC. Those highlighted in yellow show the typical expected expression pattern upon induction. Those highlighted in red and green were showing variable patterns of expression, which has to be knocked down or over expressed respectively, for efficient reprogramming.



3a



3b

Figure 3: Quantitative PCR validation of RP gene expression during HDF reprogramming and at different passages of established HDF derived iPSCs: (**3a**) Real-time PCR validation of selected RP gene expression during various days reprogramming of HDF in comparison with HDF and established iPSCs (CR5 & BC1) showing peak expression in intermediate stages of reprogramming. (**3b**) Real-time PCR validation of selected RP genes in established hiPSC lines derived from HDFs at different passages (P-5, P-27, P-43, P-65 and P-71), showing dynamic patterns of expression. Values are normalized to *ACTIN-B* and fibroblasts (P-5)- ddCt method (see methods for details).

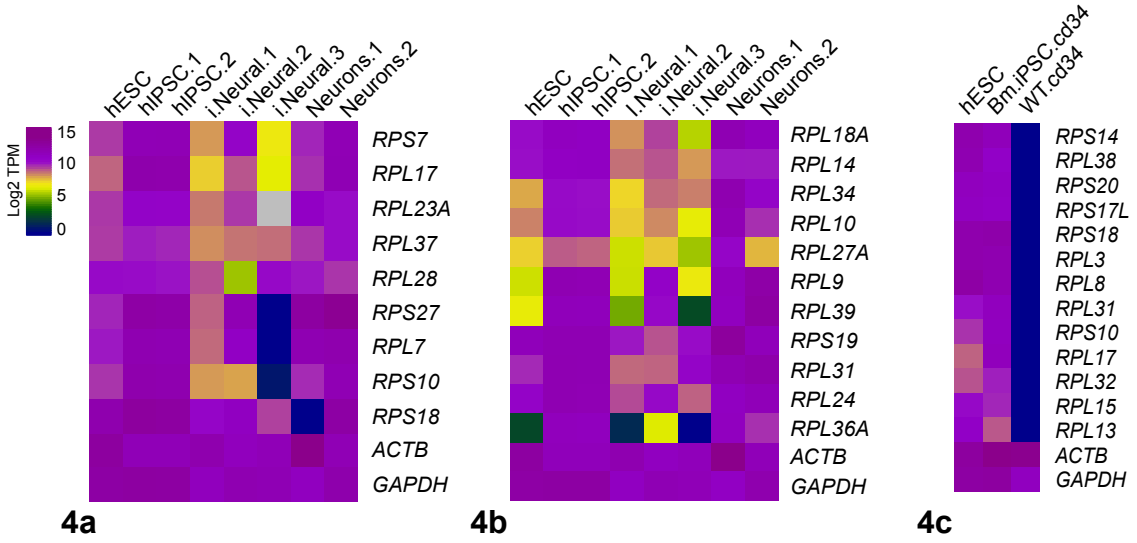


Figure 4: **Dynamic expression of RP genes among native and iPSCs derived specialized cells**: (**4a**) RP genes which showed pattern-8 of expression during somatic cell reprogramming, showing lower levels of expression in iPSCs derived neural cells than that of its *in vivo* neurons. Expression levels of RP genes in human ESCs and human iPSCs were shown for comparison. (**4b**) RP genes which do not follow pattern-8 of expression during somatic cell reprogramming, showing much lower levels of expression in iPSCs derived neural cells than that of its *in vivo* neurons. Expression levels of RP genes in human ESCs and human iPSCs were shown for comparison. (**5c**) RP genes which show relatively higher levels of expression in iPSCs derived CD4+ve cells than their *in vivo* counterparts. Human ESCs and human iPSCs were shown for comparison.

	Sense	Antisence
ACTIN-B	GACGACATGGAGAAAATCTG	ATGATCTGGGTCATCTTCTC
RPL7	CCAATTTTGTAGAAGGTGGAG	TCATGGTAGACACCTTAGTTC
RPL15	GTGCATACAAGTACATCCAG	TATAACGTAACCTTGCTTGG
RPL17	TACTTTCCTCTAGGTGATCTG	TCACGAGTGTTCTTAAAGTG
RPL23A	AAACAAGCTTGACCACTATG	GTCATACAGCTTCTTCACAG
RPL28	TCAAGAGGAATAAGCAGACC	GAAAAACTCACTCGGATCTC
RPL37	GAAAGTATAACTGGAGTGCC	GGTTTAGGTGTTGTTCCTTC
RPS3	CAAGAAGAGGAAGTTTGTCG	GTTCTGGTGGCTAAGATAATG
RPS3A	ATGGCAGACAATGATTGAAG	GTCTTCCGTATCTGATTGTTG
RPS6	AGAATGGAAGGGTTATGTGG	CTCTTTCTTTCTCCAGTTCTC
RPS9	AAGAGCTGAAGCTGATCG	TGGGTCCTTCTCATCAAG
RPS18	CAGAAGGATGTAAAGGATGG	TATTTCTTCTTGGACACACC
RPS23	AGAAGTGGCATGATAAACAG	CAACTCCTACTTTTTCCAGC
RPS27	AAAAGCAAGGCTTACAGAAG	TTATTGAGATGGTTTCCCAC
RPS29	AATATGGCCTCAATATGTGC	TCTTTTTGATGATCTTGGGC

Supplementary Table 1: List of RP gene specific primers used in quantitative PCR during different stages of reprogramming HDFs and different passages of established HDF derived iPSCs.

Name	Description	Source
hESC	Human embryonic stem cells	GSM1536664
hiPSC1	HDF derived iPSC	GSM2108668
hiPSC2	HDF derived iPSC	GSM2108669
i-neural_1	Neuronal cells derived from iPSC of HDF	GSM1536672
i-neural_2	Neurons derived from iPSC of HDF	GSM2427821
i-neural_3	Neural cells derived from iPSC of HDF	GSM2507484,GSM2507485,GSM2507486
Neurons1	Neurons	GSM1585614,GSM1585615
Neurons2	human peripheral neurons mock sample	GSM2339848
Bm-iPSC-CD34	CD34+ cells derived from iPSC of bone marrow cells	GSM1464525,GSM1464526
WT-CD34	CD34+ cells from healthy donor	GSM2754182

Supplementary Table 2: List of publicly available RNA-seq data sets from Gene expression omnibus (GEO) that were used in this study, their origin of cell-types and their respective GEO accession numbers.

Gene	hESC	hiPSC-1	hiPSC-2	i-Neural-1	i-Neural-2	i-Neural-3	Neurons-1	Neurons-2	Bm-iPSC-CD34	WT-CD34
ACTB	12.68192111	11.50717485	11.52851219	11.93661514	11.23722103	11.49425519	13.60993402	11.58824572	13.566484	13.35445508
GAPDH	12.43018416	12.54429533	12.5276164	11.20361471	11.79108357	11.66080207	11.10953775	12.04365795	12.58865491	11.10953732
PSMB2	5.850337821	8.01933862	8.019527054	6.150835066	4.636752493	7.895221758	7.000533374	5.938264954	5.982765463	0
RPL10	8.793211866	10.44507805	10.31591721	7.885425805	8.700637732	7.050871098	11.7500587	9.91324785	10.15894413	0
RPL10A	11.1604784	11.44353007	11.34511543	9.591576971	10.06192311	9.107842978	9.38198084	10.86643344	11.03939204	10.61136178
RPL10L	0	0	0	0	-3.002269447	-3.321389392	-2.931728739	0	0	0
RPL11	11.86727956	12.03488981	11.96262121	10.62257709	10.88343167	11.6318421	10.48294912	11.75062463	11.44735965	12.61162262
RPL12	8.938322544	12.11829052	11.90213504	7.378016471	9.987999467	9.165445633	10.16531284	10.74491381	11.06996996	8.761891098
RPL13	10.74436053	11.74019903	11.66622482	9.756050293	7.608493919	9.644875715	8.353780904	8.861150733	9.018965147	0
RPL13A	10.13733631	11.06429069	10.92489835	8.424760716	9.411109295	9.374346397	11.48579487	8.96872904	11.89274771	9.323567264
RPL14	10.26874488	11.09739936	11.13668682	9.035192648	9.396751914	8.519213716	10.18051006	10.16998814	10.77008158	10.47483994
RPL15	10.29095993	10.85326938	10.74455034	9.978676318	8.937759174	9.779747348	10.974262	10.05149484	9.756222713	0
RPL17	8.86585351	12.05494509	11.98500715	7.451781571	9.082958659	6.505865091	9.643043821	11.67229555	11.25473044	0
RPL18	11.58618292	11.45947313	11.25111316	10.28991054	9.180544379	11.0031506	10.42176989	10.70500525	10.79130129	12.33417183
RPL18A	10.34747795	11.27383661	11.16849256	8.596680814	9.602035201	6.346313544	11.80812613	11.32449559	11.39478153	8.436023824
RPL19	11.50107829	11.72948387	11.60254697	10.41856326	10.30430804	10.36755296	11.14878922	11.18832671	11.67308377	11.82639721
RPL21	8.733739246	9.45395904	9.449145337	7.447708841	10.434117	0	11.82117788	-3.86689779	11.29794224	1.693844604
RPL22	8.255798614	9.771282079	9.748403462	7.568431183	8.492531125	7.92673585	8.538399239	9.052344208	8.659853221	7.550993061
RPL22L1	4.458375059	5.354328691	5.522304037	3.831622894	2.958382624	3.840722159	5.451508837	4.5543732	6.077456652	6.886501399
RPL23	11.70075034	11.68548104	11.68417637	10.64322363	10.74850837	11.10915754	11.57471627	11.92285111	11.10498525	11.50847908
RPL23A	9.51531616	10.59714762	10.56139936	8.584010168	9.519991226	#N/A	10.82975786	10.15816314	10.4771916	7.87708686
RPL24	10.75331647	11.87672928	11.78141333	9.511959568	10.53788181	9.218577158	11.20716609	11.59574418	10.74412745	9.593860829
RPL26	10.76230088	11.93033677	11.86563841	9.626673446	10.86763722	9.759280118	10.56503334	12.00222456	11.28615632	11.15414184
RPL26L1	5.926556184	6.159745544	5.872467183	5.873334724	5.521297934	#N/A	7.881446729	6.908114325	6.248686627	5.663574432
RPL27	11.68149177	11.70705969	11.56304415	10.76187697	9.792031838	11.21227156	11.12893196	11.59195611	11.12602684	12.42997383
RPL27A	7.827334773	9.287280828	9.187920912	6.614731116	7.922798682	6.052442464	10.67198391	8.145954622	8.900791351	8.132239095
RPL28	10.25404434	10.14573522	9.993317054	9.159560112	5.5173305	10.29173499	9.964387322	9.560498332	10.37102516	9.953270932
RPL29	10.66940566	11.42401406	11.31568689	9.363998777	9.418282513	9.085288051	10.64450883	11.35576459	11.12222297	11.3115637
RPL3	11.94603417	12.64643185	12.56491021	10.2920084	9.760887346	10.59906686	11.67160426	11.55958828	11.81731547	0
RPL30	11.61805379	11.48063207	11.54163462	10.48149324	10.80222858	11.24679767	11.07341229	11.7391767	11.10936056	12.81648975
RPL31	9.997170238	11.86044089	11.83578753	9.125585674	9.185748526	10.71199537	11.85568885	12.22501356	11.15255104	0
RPL32	9.087331323	10.22062771	10.09689668	8.237470903	9.394120163	8.369336151	11.53171361	9.932921183	9.835876716	0
RPL34	8.32965524	10.43322628	10.27237112	7.75066651	9.111432153	8.841631251	11.94788758	10.73962158	9.513322953	10.43142193
RPL35	11.42912078	11.43027862	11.32647019	10.28775589	9.889555821	9.686455501	11.20195247	12.04110603	10.99544919	12.45667206
RPL35A	11.20419095	11.54402461	11.55253077	10.20185557	10.25668402	10.82079746	10.95103323	11.67491856	10.74251155	13.10384125
RPL36	11.53730312	11.06273368	11.05957454	10.53363999	9.040274106	10.53168186	11.12246474	11.13886894	10.65337357	12.7714511
RPL36A	3.168655294	11.42036016	11.34438786	2.188131952	6.845707173	0.883725042	11.29964125	9.902681835	9.452488362	0
RPL36AL	9.368136668	8.629739791	8.64294414	8.752085947	7.807991428	7.998784365	8.840955369	8.332235193	9.408934718	11.80554866
RPL37	9.45301581	9.893676783	9.792450448	8.303296795	8.661265386	8.746679756	9.540357192	10.12411369	9.493675351	10.21169255
RPL37A	12.84154762	12.32681113	12.25432819	12.0983207	11.01273846	11.43194925	12.0061354	12.67855134	11.54670881	14.03784502
RPL38	11.75415865	11.25212018	11.13137589	10.86184115	10.15144014	10.72327984	11.58772009	11.6176338	10.66551358	0
RPL39	7.092376783	11.49160926	11.47377177	5.513257801	10.56436327	3.26190236	11.29758516	12.59730493	10.90011206	10.2076276
RPL39L	6.762521996	6.757408162	6.780229354	4.370405485	4.904612973	4.98745523	5.55629209	5.745734187	4.821710215	1.800325213
RPL3L	0.235519855	0.845566948	0.096032484	0	-4.886720477	-3.683959461	-0.601086912	0	-2.089267338	0.961903123
RPL4	11.28597642	12.18196755	12.08917837	9.940165963	10.73707219	9.951693881	10.66651025	11.14980546	11.55718126	10.99416548
RPL41	9.968777161	12.84434278	12.67059529	8.685404354	11.52243712	-1.906351891	11.85532896	13.72603299	12.72410878	9.023529341
RPL5	10.33738641	11.78997359	11.68871936	9.105412672	10.55217134	10.6189548	10.82846243	11.28079317	10.95962528	10.90867639
RPL6	10.25333381	11.70385338	11.54783515	9.600833746	10.76495285	8.857206307	10.1820229	11.97585767	11.24530907	6.771836497
RPL7	9.942583561	11.8169115	11.71095653	8.797038383	10.86754598	0	11.73469148	11.93025034	11.61788983	6.433021589
RPL7A	9.910353962	12.29794579	12.16076877	8.80420948	10.99614043	9.806193023	10.29193051	11.83962185	11.78131668	10.05344035
RPL7L1	5.775924125	6.475278536	6.562631837	5.645724011	5.531901766	4.261066257	7.410453779	6.51602095	6.532706572	5.566586431
RPL8	12.32642442	12.1453437	12.07634347	10.79511129	9.363154508	11.31938275	10.76140397	11.31560965	11.74396973	0
RPL9	6.625815597	11.8704102	11.77466865	6.590196289	10.81363054	7.233306042	11.42829236	12.37930049	11.15265244	3.449800742
RPS10	9.565135738	11.97801736	11.86956763	8.148381282	8.020135906	0.74709035	9.718877546	11.65252762	11.09948589	0
RPS11	11.99060542	11.99817761	12.01874001	11.09746095	11.7796174	11.57315999	12.03658945	12.13431931	11.88362654	12.70795111
RPS12	11.99151078	12.18529465	12.1582818	10.31724597	9.944137376	10.43667185	9.734491511	11.93235791	11.55818152	12.52978574
RPS13	10.81263973	11.29607277	11.19235446	9.983182961	9.750201316	9.667393905	10.99744442	11.13427019	10.88041004	11.25931912
RPS14	11.90817298	11.5750851	11.4887703	10.54635771	9.675877744	11.00000636	11.62571322	11.84298335	11.33676874	0
RPS15	10.53480751	10.96075782	10.833831	9.455188479	8.973664184	7.987834304	10.73700274	11.29564324	10.79233074	10.49735861
RPS15A	10.69490443	11.95501279	11.85310968	9.577932206	10.81426589	10.61439978	10.41748841	12.20345747	11.29280188	9.318213532
RPS16	11.74539344	11.55472129	11.62614645	10.12518468	8.440469503	10.4513032	10.0026822	11.00190606	11.45437641	11.96853769
RPS17L	10.99296925	#N/A	#N/A	9.378813466	#N/A	#N/A	9.111761812	11.26691661	10.78926872	0
RPS18	11.95576758	12.68984157	12.54732827	10.463213	11.00641795	9.35402864	0	12.40712091	12.08968385	0
RPS19	11.52819814	11.82808199	11.81888196	10.18654524	9.40323831	10.38322777	12.78553002	11.51010918	11.14667686	12.40831503
RPS2	11.37168525	12.74288138	12.69961468	9.965007285	10.60009967	8.672178053	7.95633896	11.93091655	12.38675707	10.81615674
RPS20	11.15490352	11.41298569	11.30019888	9.722553927	9.905193121	10.0618949	10.37435077	10.9027395	10.91879617	0
RPS21	11.56643749	10.91277757	10.89752251	10.03784591	8.045571977	10.20330195	10.61929391	11.31919076	10.85306779	13.50403248
RPS23	8.57617074	9.618965462	9.601817095	7.921792861	8.493465987	8.014000967	11.0583709	9.089725304	9.139142019	8.187434043
RPS24	11.94661863	11.87605539	11.78737137	10.41059843	9.232098946	10.74375557	10.6867654	11.65008685	11.59626198	12.57795796
RPS25	11.228583	12.00743801	11.92664393	10.12798358	10.20773143	9.613038652	10.48399995	11.44343102	11.31090243	12.16513948
RPS26	7.206801333	8.955480359	8.981817854	7.490300707	7.915813207	#N/A	9.531091495	9.564764672	9.398583384	5.034611456
RPS27	9.80288272	12.36754855	12.2248422	8.916161483	11.63500959	0	12.57259901	13.20632866	11.6413112	0
RPS27A	9.458663701	11.29784326	11.24772893	7.997112183	9.34622017	7.819795298	11.4065316	10.39694436	10.84592515	0
RPS27L	7.238830651	7.172094228	7.172999656	7.34848867	5.452936435	6.880190022	7.158853137	8.01749279	6.146695948	10.13737071
RPS28	9.159954752	11.26555303	11.2385035	7.423977534	8.341849333	5.507628562	7.723120362	11.13310729	11.06173908	0
RPS29	12.54934045	12.03746745	12.01914514	10.98988439	7.367738121	5.518514228	11.85934429	12.84034295	11.55597429	0
RPS3	12.02702071	12.17582414	12.11785508	10.3385799	8.989314123	10.3441686	11.52141855	10.75912924	11.31745784	8.099220115
RPS3A	10.48662686	12.36890525	12.25091938	9.059611793	10.35348779	-0.048370894	11.19882766	12.33575444	11.46111873	8.41736982
RPS5	11.32857553	10.98567042	10.88564095	9.941603044	9.199413096	10.36352965	10.53664034	10.88975738	10.79581645	12.10907691
RPS6	12.2249949	12.42296591	12.43695524	10.65706111	11.2174595	11.08575295	11.12804249	11.80172558	12.07684899	12.59659697
RPS7	9.47710208	11.54883765	11.5980876	8.16395898	10.5190008	6.869532694	9.801312848	11.62293122	10.93572124	9.575578138
RPS8	11.85828616	12.33305556	12.23056778	10.63049587	10.59861796	10.82250751	10.32502373	11.76803078	11.70746483	11.84034685
RPS9	11.64041988	11.66610049	11.57196596	9.89218521	8.886105426	9.057657992	9.033994559	11.65961101	11.19410429	12.02449682

Supplementary Table 3: List of all RP genes and their log2 transformed, TPM converted expression values derived from publicly available RNA-seq data sets from hESCs, hiPSCs (1-2), neuronal cells derived from HDF iPSC (i-neural 1-3), *in vivo* neurons (1-2), CD4 cells derived from bone marrow iPSCs.