Transcriptional profiling of macrophages derived from monocytes and iPS cells identifies a conserved response to LPS and novel alternative transcription

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Abstract

Macrophages differentiated from human induced pluripotent stem cells (IPSDMs) are a potentially valuable new tool for linking genotype to phenotype in functional studies. However, at a genome-wide level these cells have remained largely uncharacterised. Here, we compared the transcriptomes of naïve and lipopolysaccharide (LPS) stimulated monocyte-derived macrophages (MDMs) and IPSDMs using RNA-Seq. The IPSDM and MDM transcriptomes were broadly similar and exhibited a highly conserved response to LPS. However, there were also significant differences in the expression of genes associated with antigen presentation and tissue remodelling. Furthermore, genes coding for multiple chemokine involved in neutrophil recruitment were more highly expressed in IPSDMs upon LPS stimulation. Additionally, analysing individual transcript expression identified hundreds of genes undergoing alternative promoter and 3′ untranslated region usage following LPS treatment representing a previously under-appreciated level of regulation in the LPS response.

Introduction

Macrophages are key cells associated with innate immunity, pathogen containment and modulation of the immune response[1,2]. Commonly used model systems for studying macrophage biology have centered on macrophage-like leukemic cell lines, primary macrophages derived from model organisms and primary human macrophages differentiated from blood monocytes. Although these cells have provided important insights into macrophage-associated biology, there are issues that need consideration. Immortalised cell lines often have abnormal genetic structures and can exhibit functional defects compared to primary cells[3,4], while multiple functional differences exist between macrophages from different species[5]. Additionally, human monocyte derived macrophages (MDMs) can be difficult to obtain in sufficient numbers for repeated experimental assays and it is currently challenging to introduce targeted mutations into their genomes, limiting their utility in genetic studies.

Recently, methods have been developed to differentiate macrophage-like cells from human induced pluripotent stem (iPS) cells that have the potential to complement current approaches and overcome some of their limitations[6,7]. This approach is scalable and large numbers of highly pure iPS-derived macrophages (IPSDMs) can be routinely obtained from any human donor following initial iPS derivation. IPSDMs also share striking phenotypic and functional similarities with primary human macrophages[6,7]. Since human iPS cells are amenable to genetic manipulation, this approach can provide large numbers of genetically modified human macrophages[7]. Previous studies have successfully used IPSDMs to model rare monogenic defects that severely impact macrophage function[8]. However, it remains unclear how closely IPSDMs resemble primary human monocyte-derived macrophages (MDMs) at the transcriptome level and to what extent they can be used as an alternative model for functional assays.

Here, we provide an in-depth comparison of the global transcriptional profiles of naïve and lipopolysaccharide (LPS) stimulated IPSDMs with MDMs using RNA-Seq. We found that their transcriptional profiles were broadly similar in both naïve and LPS-stimulated conditions. However, certain chemokine genes as well as genes involved in antigen presentation and tissue remodelling were differentially regulated between MDMs and IPSDMs. Additionally, we identified novel changes in alternative transcript usage following LPS stimulation suggesting that alternative transcription may represent an important component of the macrophage LPS response.

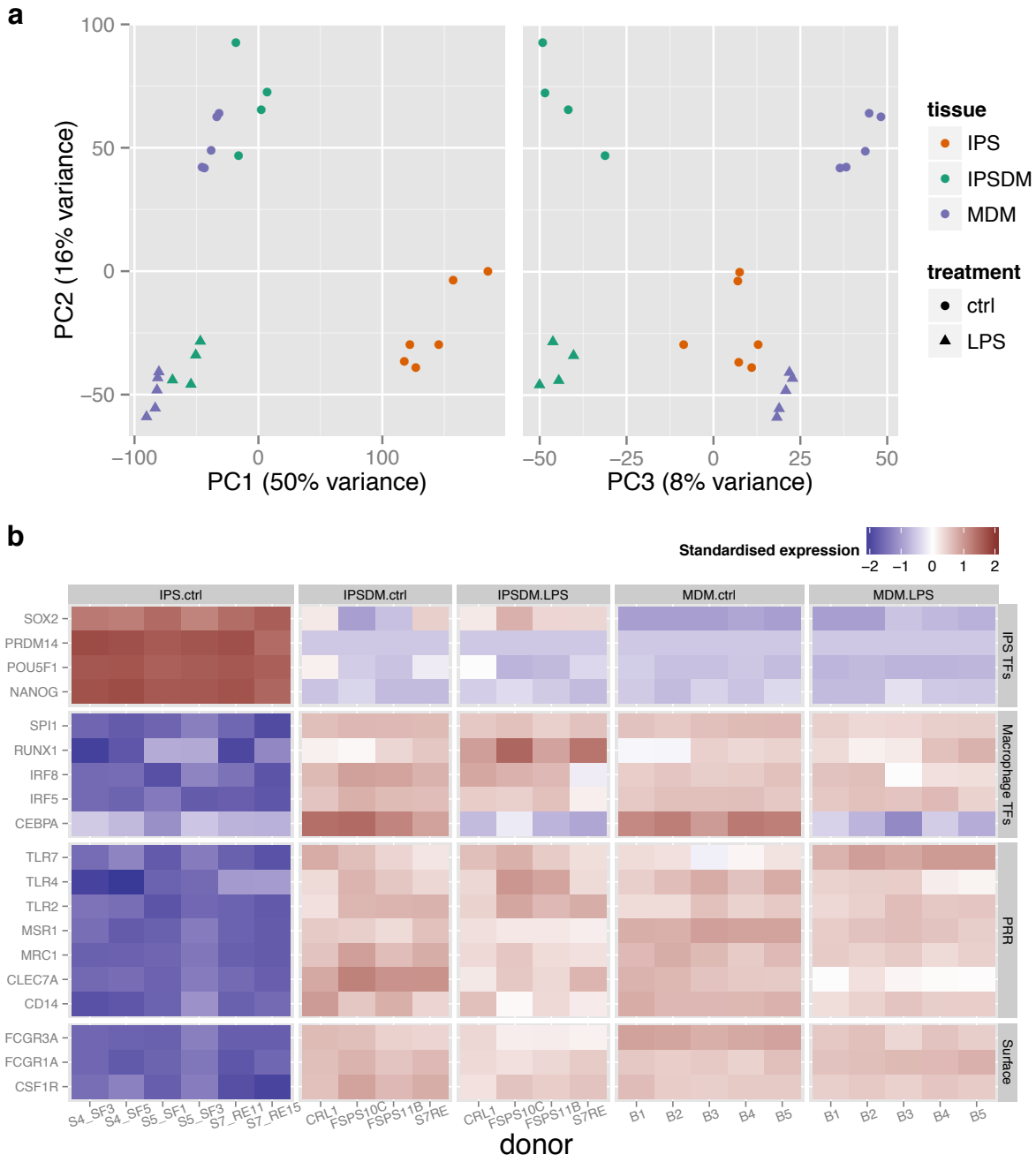

Figure 1. Gene expression variation between iPS cells, IPSDMs and MDMs. (**a**) Principal Component Analysis of expressed genes (TPM > 2) in iPS cells, IPSDMs and MDMs. (**b**) Heatmap showing the gene expression of selected iPS-specific transcription factors (TFs), macrophage specific TFs, pattern recognition receptors (PRRs) and canonical macrophage cell surface markers. Rectangles correspond to measurements from independent biological replicates.

Results

Gene expression variation between iPS cells, IPSDMs and MDMs

RNA-Seq was used to profile the transcriptomes of MDMs derived from five and IPSDMs derived from four different individuals (Methods). Identical preparation, sequencing and analytical methodologies were used for all samples. Initially, we used Principal Component Analysis (PCA) to generate a genome-wide

overview of the similarities and differences between naïve and LPS-stimulated IPSDMs and MDMs as well as undifferentiated iPS cells. The first principal component (PC1) explained 50% of the variance and clearly separated iPS cells from all macrophage samples (Fig. 1a) illustrating that the transcriptome of IPSDMs is much more similar to that of MDMs than of undifferentiated iPS cells. This was further confirmed by high expression of macrophage specific markers and low expression of pluripotency factors in IPSDMs (Fig. 1b). The second PC separated naïve cells from LPS-stimulated cells and explained 16% of the variance, while the third PC, explaining 8% of the variance, separated IPSDMs from MDMs. The principal component that separated IPSDMs from MDMs (PC3) was different from that separating macrophages from iPS cells (PC1). Since principal components are orthogonal to one another, this suggests that the differences between MDMs and IPSDMs are beyond the simple explanation of incomplete gene activation or silencing compared to iPS cells. Furthermore, genes contributing most strongly to the first and third principal components were enriched for different Gene Ontology categories (Supplementary Fig. S1).

Table 1: Selection of enriched Gene Ontology terms and KEGG pathways from different groups of differentially expressed genes. Full results are given in Supplementary Table S4.

Upregulated in LPS response			
Term ID	**Domain**	**Term name**	**p-value**
GO:0045087	BP	innate immune response	7.31E-45
GO:0009617	BP	response to bacterium	2.42E-28
GO:0032496	BP	response to lipopolysaccharide	4.38E-28
KEGG:04668	ke	TNF signaling pathway	1.71E-20
KEGG:04064	ke	NF-kappa B signaling pathway	3.56E-14
Downregulated in LPS response			
Term ID	**Domain**	**Term name**	**p-value**
GO:0005096	MF	GTPase activator activity	1.01E-09
GO:0007264	BP	small GTPase mediated signal transduction	3.14E-09
More highly expressed in MDMs compared to IPSDMs			
Term ID	**Domain**	**Term name**	**p-value**
GO:0050778	BP	positive regulation of immune response	1.97E-21
GO:0003823	MF	antigen binding	2.55E-18
GO:0005764	CC	lysosome	1.42E-17
GO:0034341	BP	response to interferon-gamma	2.17E-16
GO:0042611	CC	MHC protein complex	3.67E-16
KEGG:04612	ke	Antigen processing and presentation	3.47E-13
KEGG:04145	ke	Phagosome	2.46E-11
More highly expressed in IPSDMs compared to MDMs			
Term ID	**Domain**	**Term name**	**p-value**
GO:0030198	BP	extracellular matrix organization	3.05E-45
GO:0016477	BP	cell migration	1.50E-40
GO:0001568	BP	blood vessel development	4.89E-36
GO:0016337	BP	cell-cell adhesion	6.27E-25
GO:0001525	BP	angiogenesis	1.34E-24

Differential expression analysis of IPSDMs vs MDMs

Although PCA provides a clear picture of global patterns and sources of transcriptional variation across all genes in the genome, important signals at individual genes might be missed. To better understand transcriptional changes at the gene level we used a two factor linear model. The model included an LPS effect, capturing differences between unstimulated and stimulated macrophages and a macrophage type effect capturing differences between MDMs and IPSDMs. Our model also included an interaction term that identified genes whose response to LPS differed between MDMs and IPSDMs. We defined significantly differentially expressed genes as having a fold-change of >2 between two conditions using a p-value threshold set to control our false discovery rate (FDR) to 0.01.

Using these thresholds, we identified 2977 genes showing a significant macrophage type effect. Among these genes, 2080 gene were more highly expressed in IPSDMs and 897 were more highly expressed in MDMs (Fig. 2a). Genes that were more highly expressed in MDMs such as HLA-B, LYZ, MARCO and HLA-DRB1 (Fig. 2c), were significantly enriched for antigen binding, phagosome and lysosome pathways (Table 1, Supplementary Table S4). This result is consistent with a previous report that MDMs have higher cell surface expression of MHC-II compared to IPSDMs.[6,7] Genes that were more highly expressed in IPSDMs, such as MMP2, VEGFC and TGFB2 (Fig. 2c) were significantly enriched for cell adhesion, extracellular matrix, angiogenesis, and multiple developmental processes (Table 1, Supplementary Table S4).

In the LPS response we identified 2638 genes that were significantly differentially expressed across MDMs and IPSDMs, of which 1525 genes were upregulated while 1113 were downregulated. As might be expected, Gene Ontology and KEGG pathway analysis revealed large enrichment for terms associated with innate immune and LPS response, NF-κB and TNF signalling (Table 1, Supplementary Table S4). We also identified 569 genes whose response to LPS was significantly different between IPSDMs and MDMs. The majority of these genes (365 genes) were up- or downregulated in both macrophage types but the magnitude of change was significantly different. Overall, we found that the fold change of the genes that responded to LPS was highly correlated between MDMs and IPSDMs ($r = 0.82$, Fig. 2b) indicating that the LPS response in these two macrophage types is broadly conserved. The behaviour of some canonical LPS response genes is illustrated in Fig. 2d.

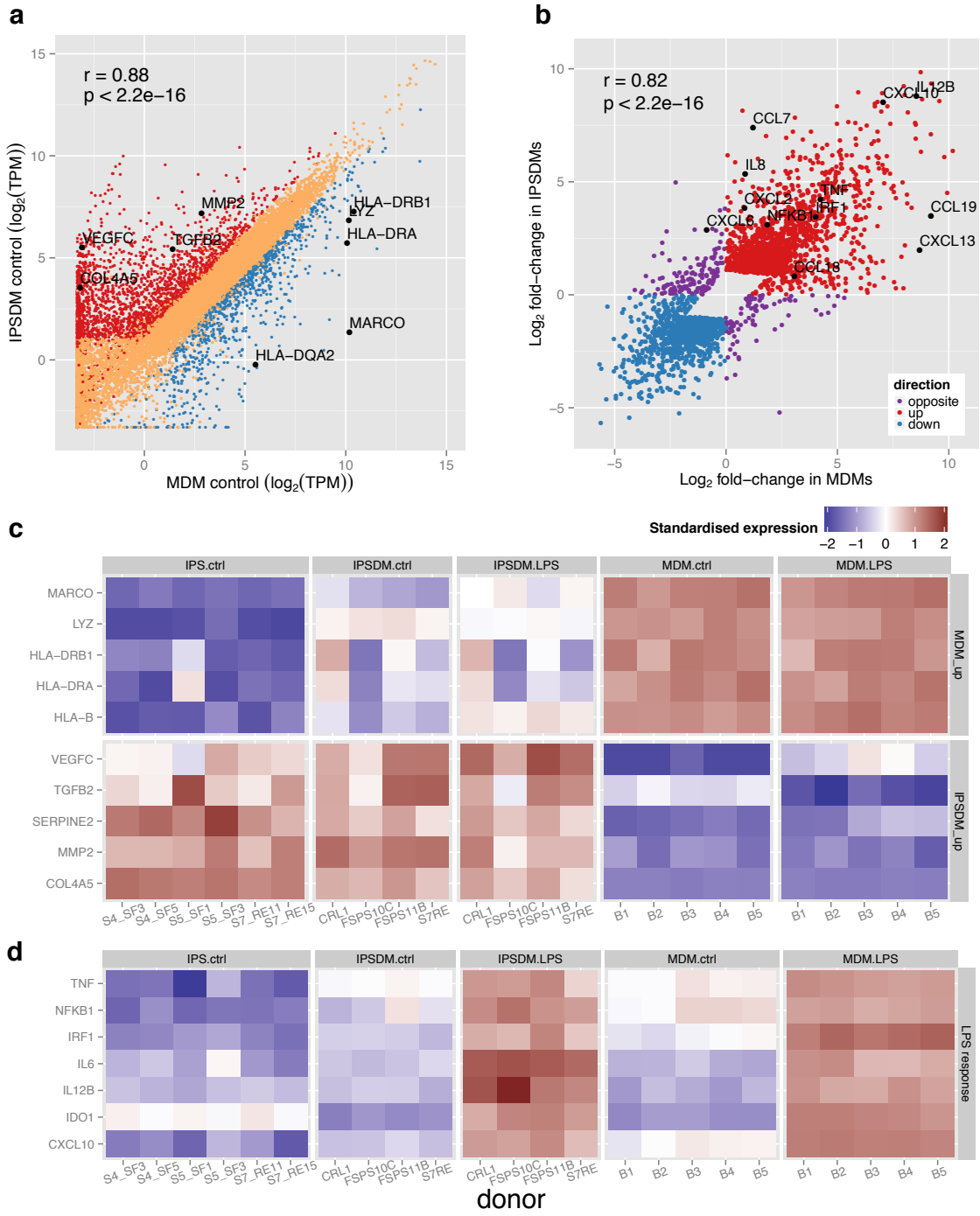

Figure 2. Differential expression analysis of IPSDMs vs MDMs. **(a)** Scatter plot of gene expression levels between MDMs and IPSDMs. Genes that are significantly more highly expressed in IPSDMs are shown in red and genes that are significantly more highly expressed in MDMs are shown in blue. **(b)** Scatter plot of gene expression fold-change in response to LPS between MDMs (x-axis) and IPSDMs (y-axis). Only genes with significant LPS or interaction term in the linear model are shown. Genes where LPS response is in opposite directions between MDMs and IPSDMs are highlighted in purple. **(c)** Heat map of genes differentially expressed between MDMs and IPSDMs. Representative genes from significantly overrepresented Gene Ontology groups (Table 1) include antigen presentation (HLA genes), lysosome formation (LYZ), angiogenesis (VEGFC, TGFB2), and extracellular matrix (SERPINE2, MMP2 COL4A5). The same genes are also marked in panel a. **(d)** Heatmap of example canonical genes upregulated in LPS response.

IL8 and CCL7 mRNAs were particularly upregulated in IPSDMs compared to MDMs (Fig. 2b). Consequently, we looked at the response of all canonical chemokines in an unbiased manner[9]. Interestingly, we observed relatively higher induction of further CXC subfamily monocyte and neutrophil attracting chemokines in IPSDMs (Fig. 3). Moreover, five out of seven CXCR2 ligands[9] were more strongly induced in IPSDMs (FDR < 0.1, fold-change difference between MDMs and IPSDMs > 2) which is significantly more than is expected by chance (Fisher's exact test p=4.531e-06) (Fig. 3). These genes were also expressed at substantial levels (TPM > 100, Supplementary Table S6), with IL8 being one of the most highly expressed gene in IPSDMs after LPS stimulation. On the other hand, MDMs displayed relatively higher induction of three chemokines involved in attracting B-cells, T-cells and dendritic cells (CCL18, CCL19, CXCL13) (Fig. 3).

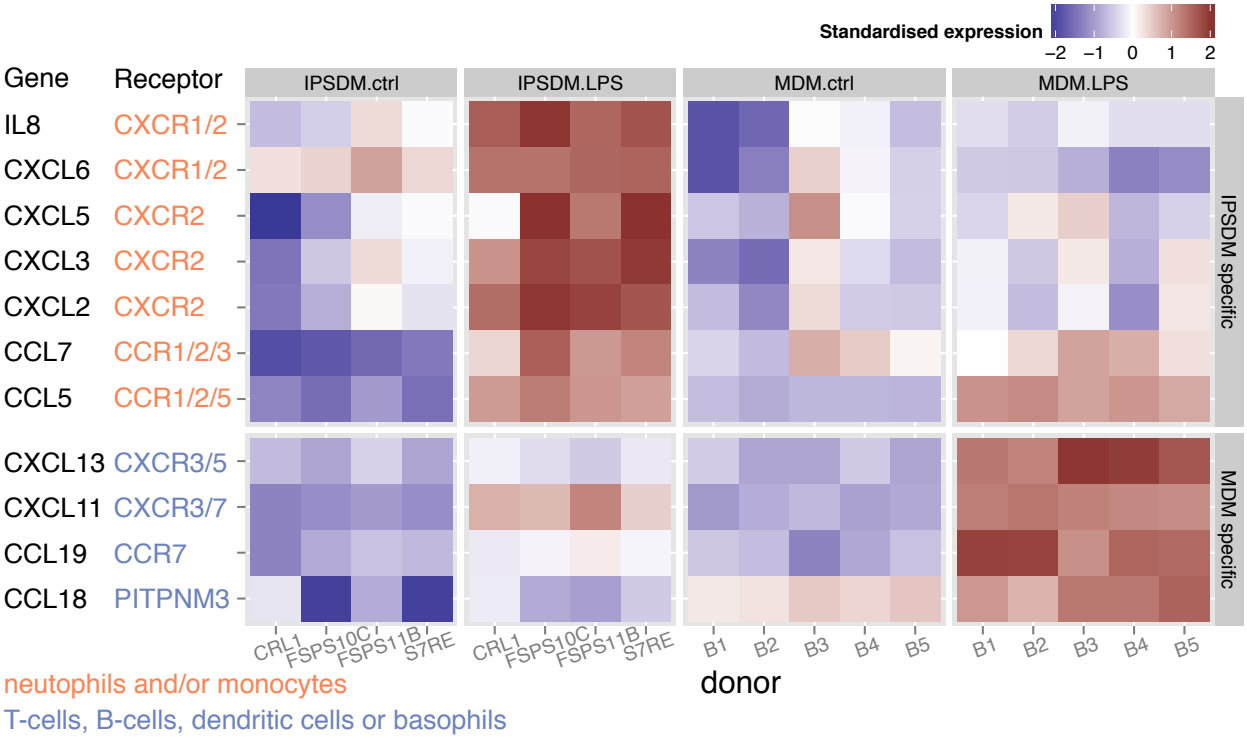

Figure 3. Chemokine genes that were particularly upregulated in either IPSDMs or MDMs in LPS response. Their annotated receptors and target cell types were taken from the literature[9,22]. Mean absolute expression values are in shown in Supplementary Table S6.

Global variation in alternative transcript usage

Many human genes express multiple transcripts that can differ from each other in terms of function, stability or sub-cellular localisation of the protein product[10,11]. Considering expression only at a whole gene level can hide some of these important differences. Therefore, we sought to quantify how similar were naïve and stimulated IPSDMs and MDMs at the individual transcript expression level. Here, we first used mmseq[12] to estimate the most likely expression of each annotated transcript that would best fit the observed pattern of RNA-Seq reads across the gene. Next, we calculated the proportion of each transcript by dividing transcript expression by the overall expression level of the gene, only including genes that were expressed over two transcripts per million (TPM)[13] in all experimental conditions (8284 genes). Since the proportions of all transcripts of a gene sum up to one and most genes express one dominant transcript[14], we used the proportion of the most highly expressed transcript to represent the transcriptional make-up of the gene. In this context and similarly to gene level analysis, the first PC explained 31% of the

variance and clearly separated IPS cells from macrophages (Fig. 4a). However, here the second PC (11% of variance) not only separated unstimulated from stimulated cells but also IPSDMs from MDMs. One interpretation of this result is that the changes in transcript usage between IPSDMs and MDMs, to some extent, also resemble those induced in the LPS response. Further analysis (below) highlighted that much of this variation can be explained by changes in 3′ untranslated region (UTR) usage.

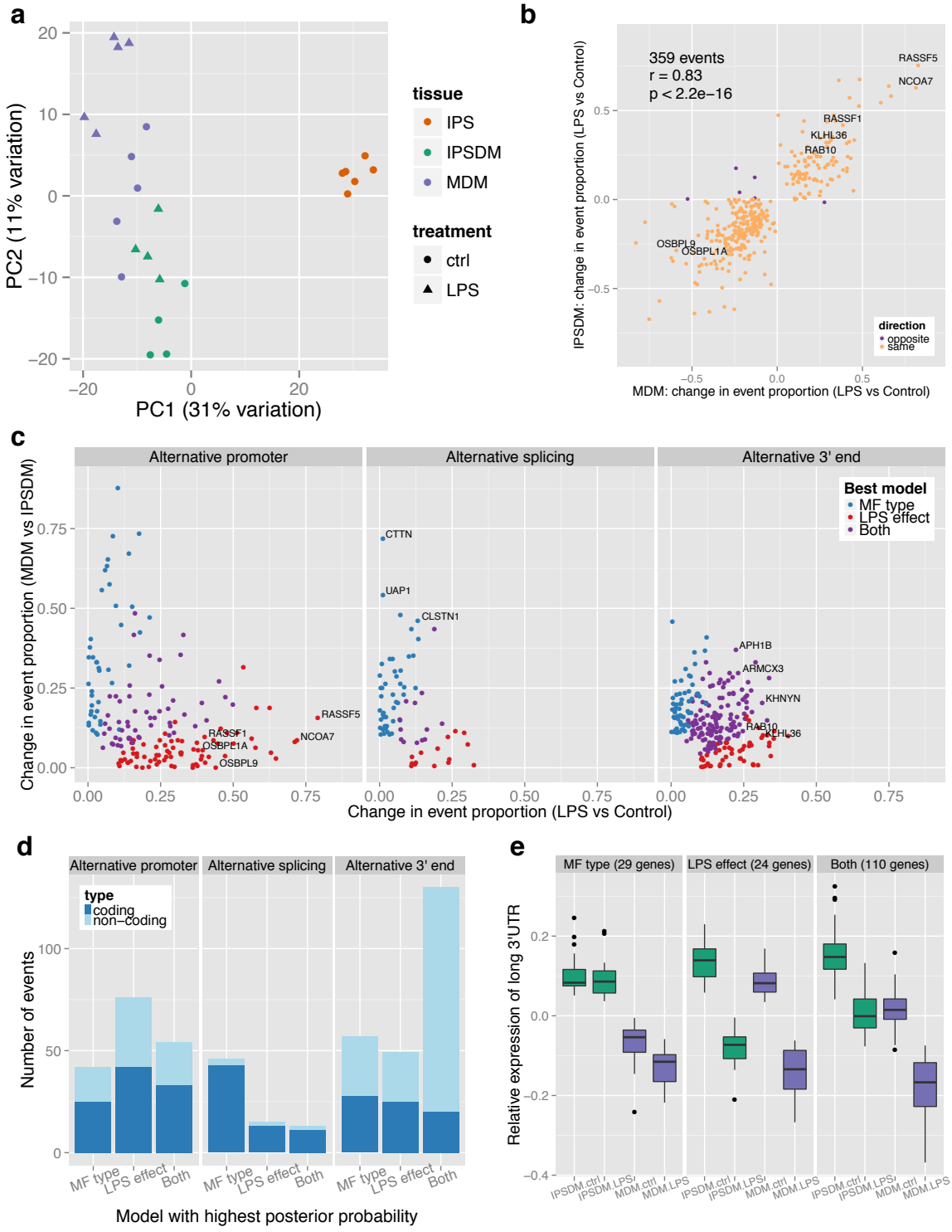

Figure 4. Alternative transcription in IPSDMs and MDMs. (**a**) Principal component analysis of relative transcript proportions in IPS cells, IPSDMs and MDMs. Only genes with mean TPM > 2 in all conditions were included. (**b**) Alternative transcription events detected in LPS response. Each point corresponds to an alternative transcription event and shows the absolute change in the proportion of the most highly expressed transcript (across all samples) in LPS response in MDMs (x-axis) and IPSDMs (y-axis). (**c**) All detected alternative transcription events were divided into three groups based on whether they affected alternative promoter, alternative splicing or alternative 3′ end of the transcript. For each event, we plotted its change in proportion in LPS response (x-axis) against its change between macrophage types (y-axis). The events are coloured by the most parsimonious model of change selected by mmseq: LPS effect (difference between naïve and LPS-stimulated cells only); macrophage (MF) type (difference between IPSDMs and MDMs only); both (data support both MF type and LPS effects). (**d**) Number of alternative transcription events form panel C grouped by position in the gene (alternative promoter, alternative splicing, alternative 3′ end) and most parsimonious model selected by mmseq. (**e**) Relative expression of long alternative 3′ UTRs in genes showing a change between IPSDM and MDMs (MF type), between naïve and LPS-stimulated cells (LPS effect) and for genes showing both types of change.

Identification and characterisation of alternative transcription events

Alternative transcription can manifest in many forms, including alternative promoter usage, alternative splicing and alternative 3′ end choice, each likely to be regulated by independent biological pathways. Thus, we sought to characterise and quantify how these different classes of alternative transcription events were regulated in the LPS response, and between MDMs and IPSDMs. Using a linear model implemented in the mmdiff[15] package followed by a series of downstream filtering steps (Methods) we identified 504 alternative transcription events in 485 genes. Out of those, 162 were distinct in the LPS response, 145 were distinct between macrophage types and 197 changed in both comparisons (Fig. 4d). Focussing on the 359 events identified either in the LPS response or in both comparisons, we found that the magnitude of change was highly correlated between MDMs and IPSDMs (Pearson r = 0.83) (Fig. 4b), further confirming that the LPS response in both macrophage types is conserved. Perhaps surprisingly, although the transcriptional response to LPS at the whole gene level is relatively well understood, the effect of LPS on transcript usage has remained largely unexplored. Therefore we decided to investigate the types of alternative transcription events identified in the LPS response as well as between MDMs and IPSDMs.

Most protein coding changes in the LPS response were generated by alternative promoter usage (Fig. 4c-d). In total, we identified 180 alternative promoter events, 51 of which changed the coding sequence by more than 100 bp in the LPS response (Supplementary Table S5, Supplementary Fig. S2). Strikingly, alternative promoter events had larger effect sizes than other events and they often changed the identity of the most highly expressed transcript of the gene (Fig. 4c). Strong activation of an alternative promoter in the NCOA7 gene is illustrated in Fig. 5a. More examples can be found in Supplementary Fig. S2.

We also observed widespread alternative 3′ end usage both in the LPS response as well as between MDMs and IPSDMs (Fig. 4c-d). In contrast to alternative promoters, most of the 3′ end events only changed the length of the 3′ UTR and not the coding sequence (Fig. 4d). Changes in 3′ UTR usage were also strongly asymmetric with IPSDMs expressing transcripts with longer 3′ UTRs than MDMs and unstimulated cells expressing relatively longer 3′ UTRs than stimulated cells (Fig. 4e, Fig. 5b). Notably, the observed pattern of decreased 3′ UTR length from unstimulated IPSDMs to stimulated MDMs corresponded to the second principal component of relative transcript expression (Fig. 4a). Consistent with that, we found that genes with significant 3′ UTR events were enriched among the genes that contributed strongly to PC2 (Supplementary Fig. S3).

Finally, we detected only a small number of alternative splicing events influencing middle exons, most of which occurred between MDMs and IPSDMs rather than in the LPS response (Fig. 4c-d). Three of the events with largest changes in relative expression affected cassette exons in genes UAP1 (Fig. 5c), CTTN and CLSTN1 (Supplementary Fig. S4). Interestingly, the inclusion of these exons has previously been

shown to be regulated by RNA-binding protein RBFOX2 that was significantly more highly expressed in IPSDMs (Fig. 5c)[16,17].

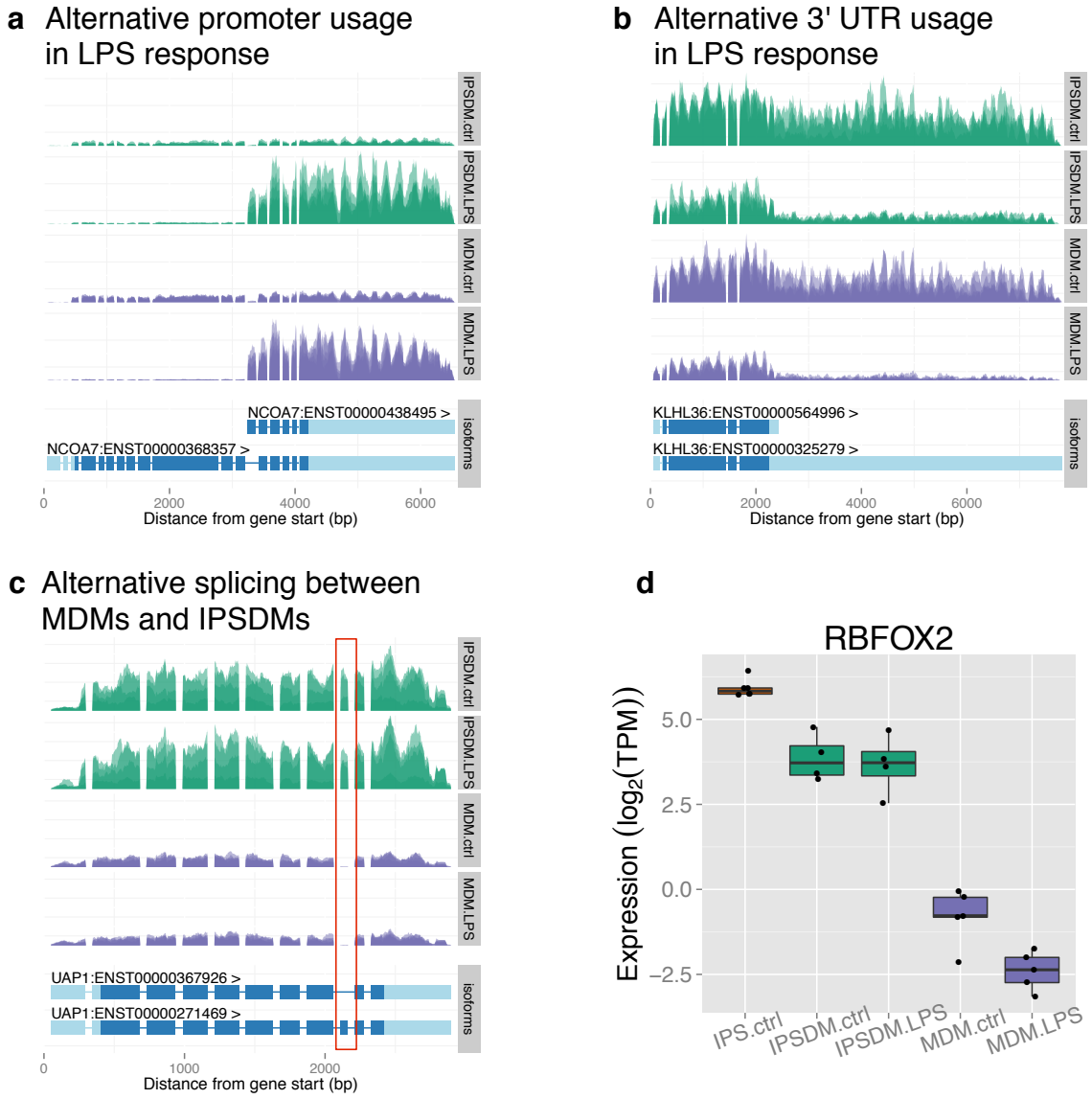

Figure 5. Examples of alternative transcript usage. Each plot shows normalised read depth across the gene body in IPSDMs (green) and MDMs (purple) with gene structure in the panel beneath each plot. Introns have been compressed relative to exons to facilitate visualisation. (**a**) Example of alternative promoter usage in LPS response. (**b**) Examples of 3′ UTR shortening in LPS response. (**c**) Example of alternative splicing between MDMs and IPSDMs. The alternatively spliced exon is marked with the red rectangle. (**d**) Expression of RBFOX2 gene in iPS cells, IPSDMs and MDMs.

Discussion

In this study, we used high-depth RNA-Seq to investigate transcriptional similarities and differences between human monocyte and iPS-derived macrophages. Our principal findings are that the transcriptomes of naïve and LPS stimulated MDMs and IPSDMs are broadly similar both at the whole gene and individual transcript levels. Although we have only examined steady-state mRNA levels, conservation of transcriptional response to LPS implies that the major components of signalling on

protein level that coordinate this response must be similarly conserved. We did, however, also observe intriguing differences in expression in specific sets of genes, including those involved in tissue remodelling, antigen presentation and neutrophil recruitment, suggesting that IPSDMs might possess some phenotypic differences from MDMs. Our analysis also revealed a rich diversity of alternative transcription changes suggesting widespread fine-tuning of regulation in macrophage LPS response.

There are a number of possible explanations for the differences we observed in transcription between MDMs and IPSDMs. Although our IPSDM samples could include minority populations of other cell types these were not obvious and all of our IPSDM samples were highly pure (92-99% CD14+) (Supplementary Fig. S5, Supplementary Table S1), excluding contamination as the major source of these differences. Alternatively, IPSDMs could show incomplete differentiation from iPS cells. Consistent with this hypothesis, genes that were more highly expressed in IPSDMs were often also expressed in iPS cells (Fig. 2c, Supplementary Fig. S6a) and large fraction of these genes had very low absolute expression (Supplementary Fig. S6b). Furthermore, the promoters of the upregulated genes were highly enriched for repressive H3K27me3 histone marks in CD14+ monocytes[18] (Supplementary Fig. S6c), suggesting that these genes become silenced prior to monocyte-macrophage differentiation *in vivo* and may not have been completely silenced in IPSDMs. Finally, IPSDMs might reflect a different subtype of macrophages. In support of that, higher expression of tissue remodelling and neutrophil recruitment genes has previously been associated with tissue and tumour associated macrophages[19–22]. On the other hand, higher expression of antigen binding genes in MDMs is consistent with the specialised role of monocyte-derived cells in immune regulation and antigen presentation[22–24]. Nevertheless, it is likely that the exact characteristics of IPSDMs can be shaped by the addition of cytokines and other factors during differentiation and this could be an important area for further exploration.

In addition to showing that LPS response was broadly conserved between MDMs and IPSDMs both on gene and transcript level, we also identified hundreds of individual alternative transcription events, highlighting an important, but potentially overlooked, regulatory mechanism in innate immune response. A small number of the events have known functional consequences. For example, the LPS-induced short isoform of the NCOA7 (Fig. 5a) gene is known to be regulated by Interferon β-1b and it is suggested to protect against inflammation-mediated oxidative stress[25] whereas the long isoform is a constitutively expressed coactivator of estrogen receptor[26]. Similarly, the two isoforms of the OSBPL1A gene (Supplementary Fig. S2) have distinct intracellular localisation and function[27] while the LPS-induced short transcript of the OSBPL9 gene (Supplementary Fig. S2) codes for an inhibitory isoform of the protein[28]. Thus, alternative promoter usage has the potential to significantly alter gene function in LPS response and these changes can be missed at gene level analysis.

Widespread shortening of 3′ UTRs has previously been observed in proliferating cells and cancer as well as activated T-cells and monocytes[29,30]. The functional consequences of 3′ UTR shortening are unclear, but extended 3′ UTRs are often enriched for binding sites for miRNAs or RNA-binding proteins that can regulate mRNA stability and translation efficiency[29,31]. The role of miRNAs in fine-tuning immune response is well established[32]. Furthermore, interactions between alternative 3′ UTRs and miRNAs have recently been implicated in the brain[33,34]. Therefore, it might be interesting to explore how 3′ UTR shortening affects miRNA-dependent regulation in LPS response.

In summary, we have performed an in depth comparison of an iPS derived immune cell with its primary counterpart. Our study suggests that iPS-derived macrophages are potentially valuable models for the study of innate immune stimuli in a genetically manipulable, non-cancerous cell culture system. The ability to readily derive and store iPS cells potentially enables in-depth future studies of the innate immune response in both healthy and diseased individuals. A key advantage of this model will be the ability to study the impact of human genetic variation, both natural and engineered, in innate immunity.

Methods

Samples

Human blood for monocyte-derived macrophages was obtained from NHS Blood and Transplant, UK and all experiments were performed according to guidelines of the University of Oxford ethics review committee. All IPSDMs were differentiated from four iPS cell lines: CRL1, S7RE, FSPS10C and FSPS11B. CRL1 iPS cell line was originally derived from a commercially available human fibroblast cell line and has been described before[35]. S7RE iPS cell line was derived as part of an earlier study from our lab[36]. FSPS10C and FSPS11B iPS cell lines were derived as part of the Human Induced Pluripotent Stem Cell Initiative. All iPS cell work were carried out in accordance to UK research ethics committee approvals (REC No. 09/H306/73 & REC No. 09/H0304/77).

Cell culture and reagents

IPS cells were grown on mouse embryo fibroblast feeder cells in Advanced DMEM F12 (Gibco) supplemented with 20% Knockout serum replacement (Gibco), 2mM L-glutamine, 50 IU/ml penicillin, 50 IU/ml streptomycin and 50 µM 2-mercaptoethanol (Sigma M6250). The medium was supplemented with 4 ng/ml rhFGF basic (R&D) and changed daily. Prior to passage, the cells were lifted from the tissue culture plates with 1:1 collagenase-dispase solution. Human M-CSF producing cell line CRL-10154 (ATCC) was grown in T150 flasks containing 40 ml of medium (90% minimum essential medium, 10% FBS, 2mM L-glutamine, 50 IU/ml penicillin, 50 IU/ml streptomycin). On day 9 the supernatant was sterile-filtered and stored at -80°C.

IPS cells were differentiated into macrophages following a previously published protocol[7]. Briefly, the key stages of differentiation are: i) formation of three germ layer containing embryoid bodies (EBs) from iPS cells on withdrawing FGF, ii) long term production of myeloid precursor cells from EBs in the presence of 25ng/ml IL-3 and 50ng/ml M-CSF (both R&D) and iii) terminal differentiation and maturation of myeloid precursors into mature macrophages in the presence of higher concentrations of M-CSF (100 ng/ml). In the last step we supplemented macrophage differentiation media with 20% supernatant from CRL-10154 cell line instead of recombinant M-CSF specified in the original protocol. We observed that using supernatant did not alter gene expression profile of macrophages (Supplementary Fig. S7b). Human monocytes (98% $CD14^+$, 13% $CD16^+$) were obtained from healthy donor buffy coats by 2-step gradient centrifugation[37]. The isolated monocytes were cultured for 7 days in the same macrophage differentiation medium as IPSDMs.

On day 7 of macrophage differentiation, medium was replaced with either fresh macrophage medium (without M-CSF) or medium supplemented with 2.5 ng/ml LPS (*E. coli*). After 6 hours cells were lifted from the plate using lidocaine solution (6 mg/ml lidocaine, PBS, 0.0002% EDTA), counted with haemocytometer (C-Chip) and lysed in 600 µl RLT buffer (Qiagen).

RNA extraction and sequencing

RNA was extracted with RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. After extraction, the sample was incubated with Turbo DNase at 37°C for 30 minutes and subsequently re-purified using RNeasy clean-up protocol. Standard Illumina unstranded poly-A enriched libraries were prepared and then sequenced 5-plex on Illumina HiSeq 2500 generating 20-50 million 75bp paired-end reads per sample. RNA-Seq data from six iPS cell samples was taken from a previous study[36]. Sample information together with the total number of aligned fragments are detailed in Supplementary Table S2.

Flow cytometry analysis

Approximately $1x10^6$ cells were resuspended in flow cytometry buffer (D-PBS, 2% BSA, 0.001% EDTA) supplemented with Human TruStain FcX (Biolegend) and incubated for 45 minutes on ice to block the Fc receptors. Next, cells were washed once and resuspended in buffer containing one of the antibodies or

isotype control. After 1 hour, cells were washed three times with flow cytometry buffer and immediately measured on BD LSRFortessa cell analyser. The following antibodies (BD) were used (cat no): CD14-Pacific Blue (558121), CD32-FITC (552883), CD163-PE (556018), CD4-PE (561844), CD206-APC (550889) and PE isotype control (555749). The data were analysed using FlowJo. The raw data are available on figshare (doi: 10.6084/m9.figshare.1119735).

Data analysis

Sequencing reads were aligned to GRCh37 reference genome with Ensembl 74 annotations using TopHat v2.0.8b[38]. Reads overlapping gene annotations were counted using featureCounts[39] and DESeq2[40] was used to identify differentially expressed genes. Genes with FDR < 0.01 and fold-change > 2 were identified as differentially expressed. We used g:Profiler to perform Gene Ontology and pathway enrichment analysis[41]. All analysis was performed on genes classified as expressed in at least one condition (TPM > 2) except where noted otherwise. The bedtools suite was used to calculate genome-wide read coverage[42]. All downstream analysis was carried out in R and ggplot2 was used for figures.

To quantify alternative transcript usage, reads were aligned to Ensembl 74 transcriptome using bowtie v1.0.0[43]. Next, we used mmseq and mmdiff to quantify transcript expression and identify transcripts whose proportions had significantly changed[12,15]. For each transcript we estimated the posterior probability of five models (i) no difference in isoform proportion (null model), (ii) difference between LPS treatment and controls (LPS effect), (iii) difference between IPSDMs and MDMs (macrophage type effect), (iv) independent treatment and cell type effects (both effects), (v) LPS response different between MDMs and IPSDMs (interaction effect). We specified the prior probabilities as (0.6, 0.1, 0.1, 0.1, 0.1) reflecting the prior belief that most transcripts were not likely to be differentially expressed. Transcripts with posterior probability of the null model < 0.05 were considered significantly changed.

We used two-step analysis to identify alternative transcription events from alternative transcripts. First, to identify all potential alternative promoter, alternative splicing and alternative 3′ end events in each gene, we compared the most significantly changed transcript to the most highly expressed transcript of the gene (Supplementary Fig. S8). Next, we reanalysed the RNA-Seq data using exactly the same strategy as described above (bowtie + mmseq + mmdiff) but substituted Ensembl 74 annotations with the identified alternative transcription events. This allowed us to separate the events truly supported by the data from the ones that were identified only because they were on the same transcript with a causal event (Supplementary Fig. S8). Finally, we required events to change at least 10% in proportion between two conditions to be considered for downstream analysis. The code to identify alternative transcription events from two transcripts is implemented in the reviseAnnotations R package (https://github.com/kauralasoo/reviseAnnotations). Our event-based approach is similar to the one used by MISO[44].

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Author contributions

Contribution: K.A., S.M. and D.G. designed the experiment; K.A. and C.H. performed the IPSDM experiments; F.M.E. performed the MDM experiments; K.A. analysed the data; K.A., S.M., D.G., G.D., F.P. and S.G interpreted the results; K.A., D.G. and S.M. wrote the manuscript; D.G., G.D. S.G. and F.P. supervised the research. All authors discussed the results and commented on the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Accession codes

RNA-Seq data are available from the European Genome-phenome Archive (EGA) under accession EGAS00001000563.

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