Supplementary Information

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

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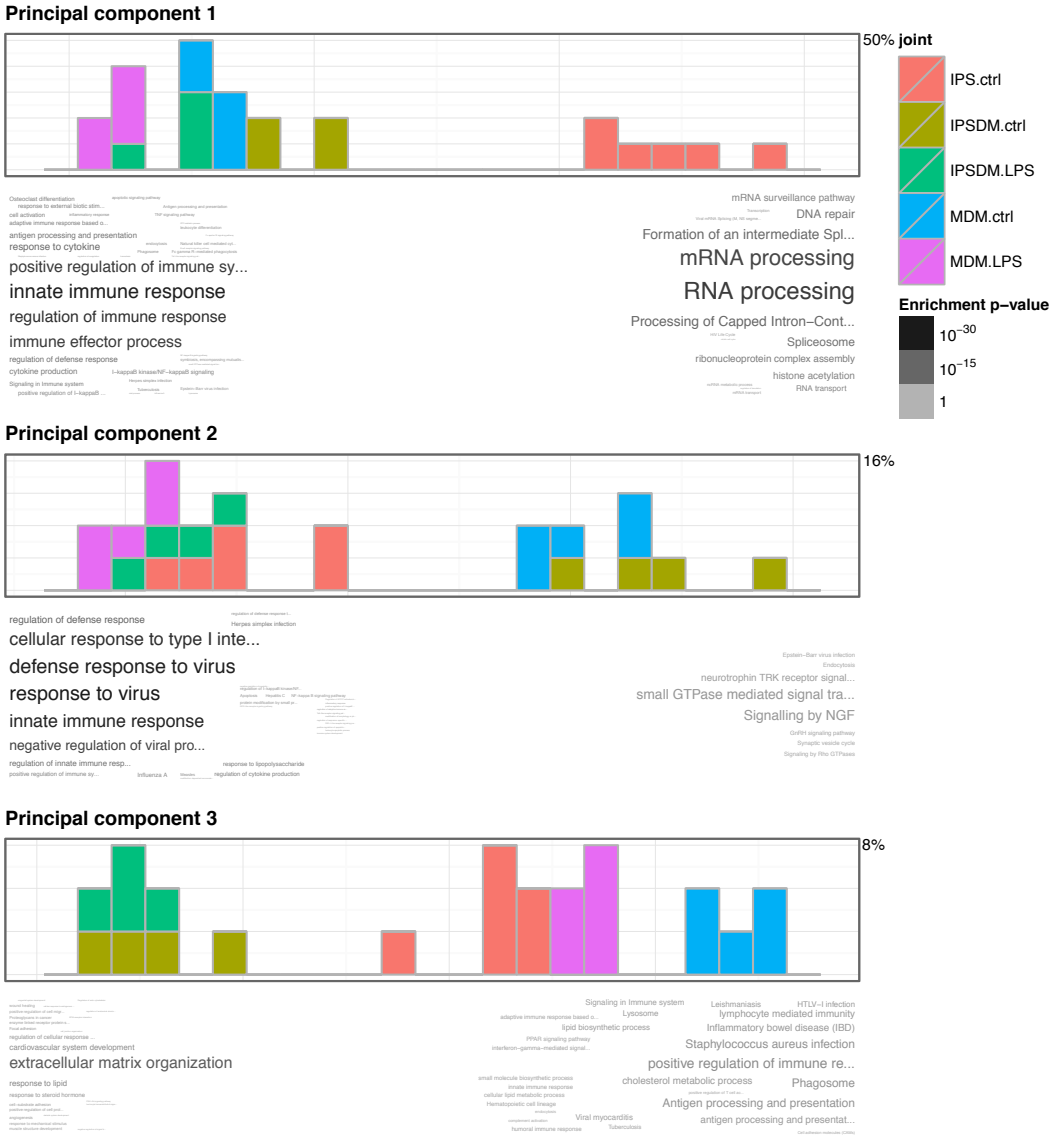
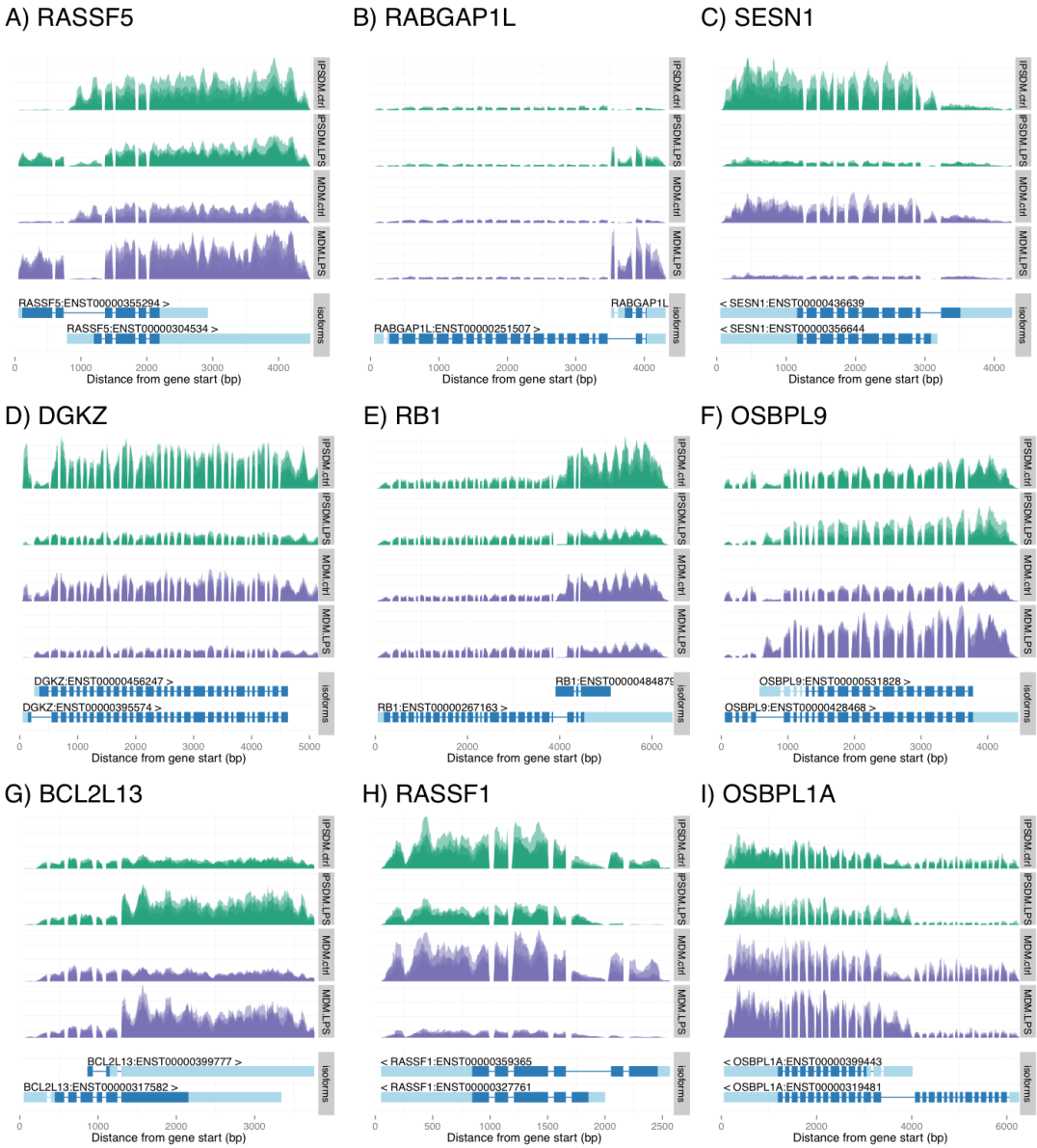
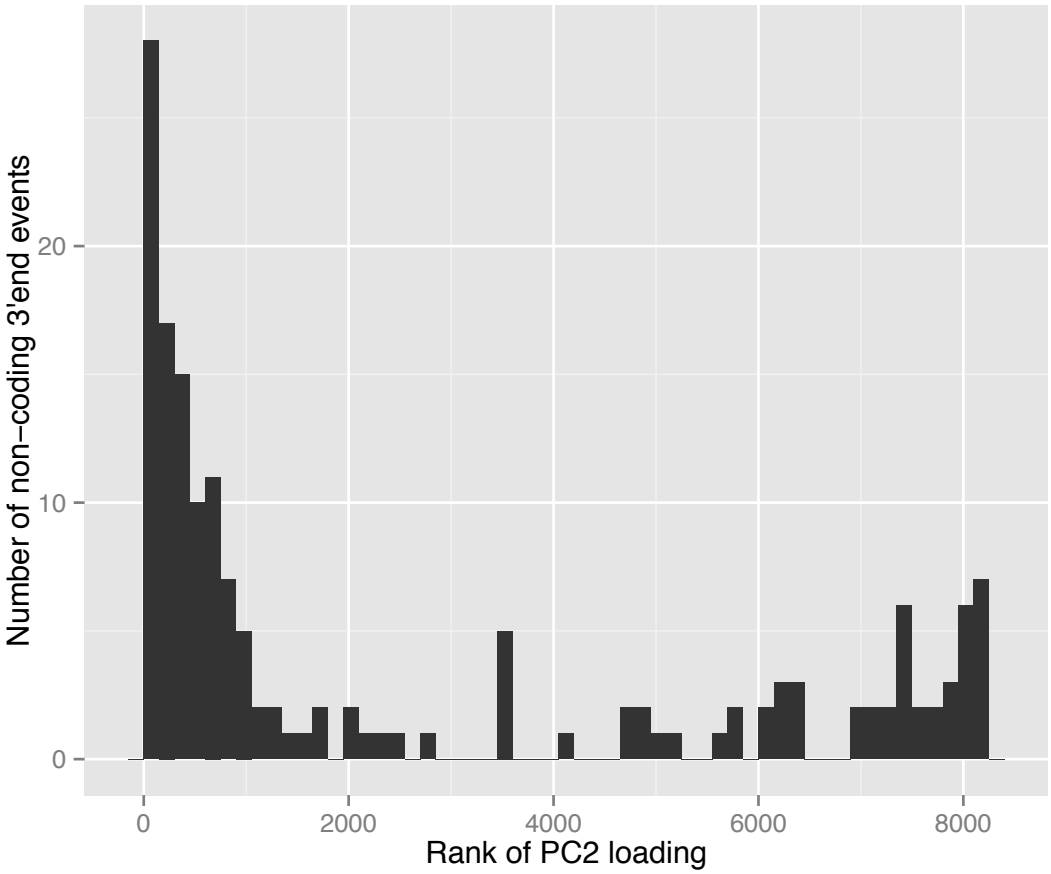


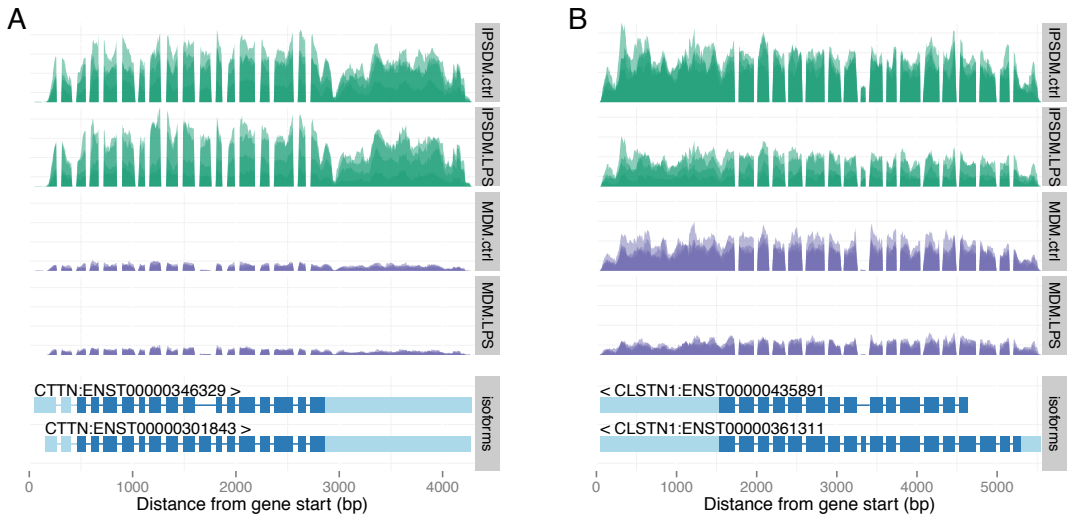
Supplementary Figure S1: Gene Ontology terms enriched in the first three principal components. PCA analysis on gene expression levels was performed as described in the main text. The results were then fed into GOsummaries R package to identify Gene Ontology categories that were enriched among genes with high loadings in each principal component.



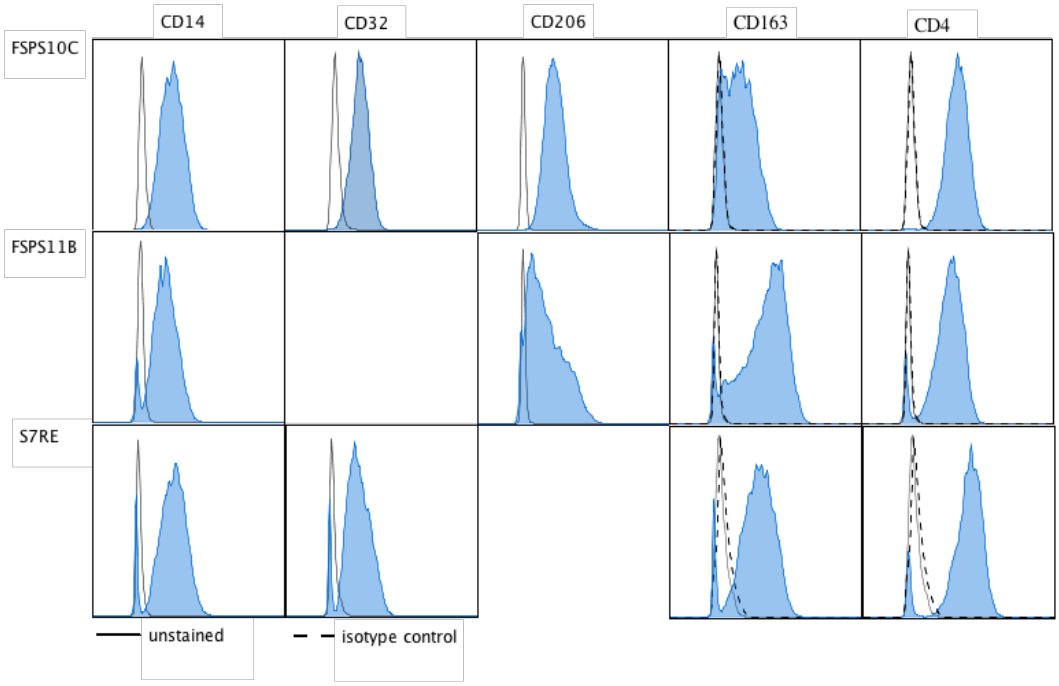
Supplementary Figure S2: Example genes showing strong alternative promoter usage in LPS response. Each panel shows normalised read depth across the gene body and flanking regions in IPSDMs (green) and MDMs (purple) with gene structure in the panel beneath each plot. Introns have been compressed relative to exons to facilitate visualisation.



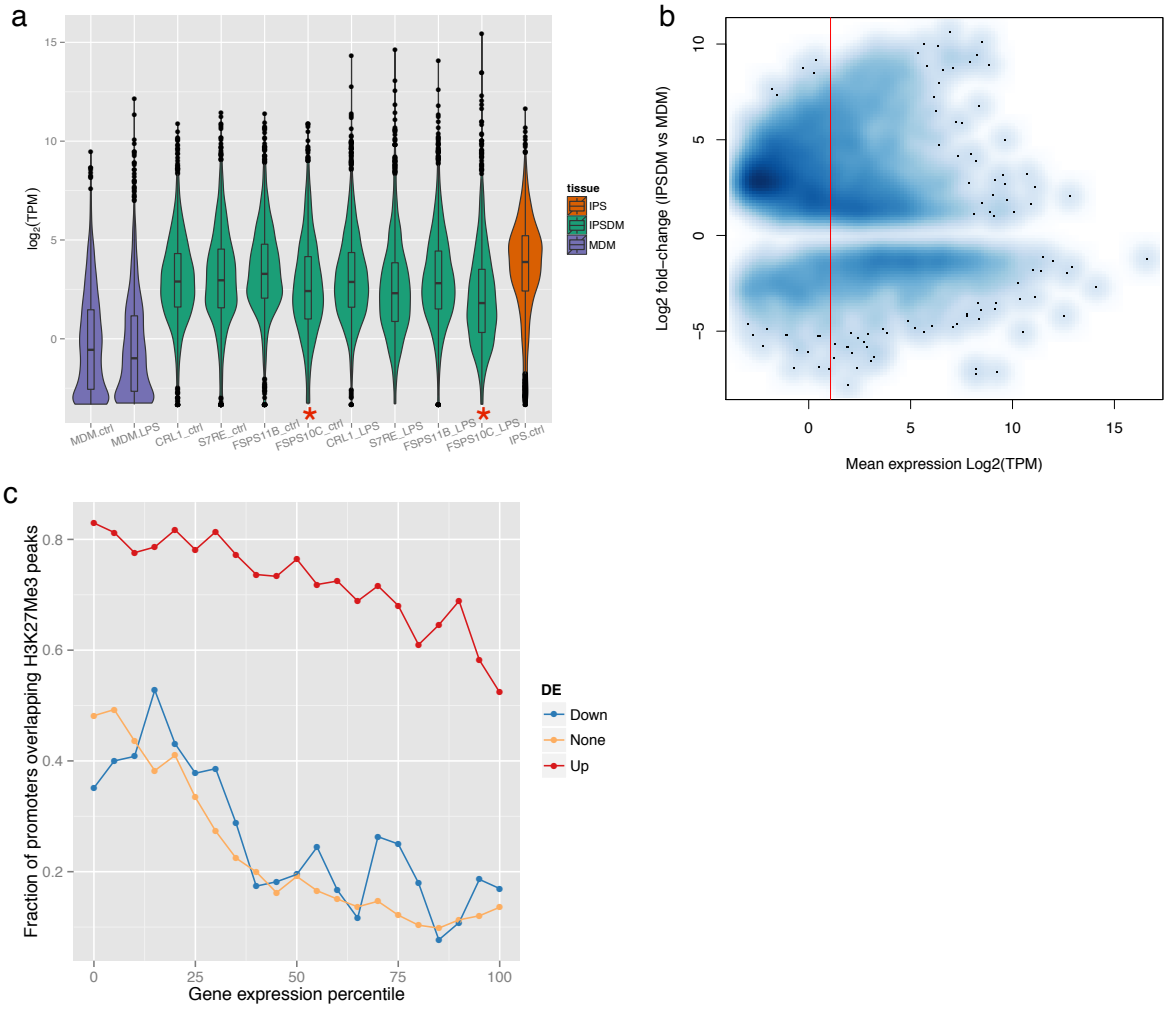
Supplementary Figure S3: Distribution of principal component 2 (PC2) weights for genes with 3' UTR changes. Principal component analysis on transcript proportions was performed as described in the main text. All genes were then ranked based on their weights in PC2. Finally the ranks for those genes that displayed 3' UTR shortening either in LPS response or between MDMs and IPSDMs (168 genes) are displayed on the histogram. If the genes with 3' UTR changes did not contribute to PC2 their ranks would be uniformly distributed.



Supplementary Figure S4: Examples of alternative splicing between MDMs and IPSDMs. (a) Read coverage plot of the CTTN gene. Middle exon is differentially included between MDMs and IPSDMs. (b) Read coverage plot of the CLSTN1 gene. Middle exon is differentially included between MDMs and IPSDMs. Each panel shows normalised read depth across the gene body and flanking regions 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.



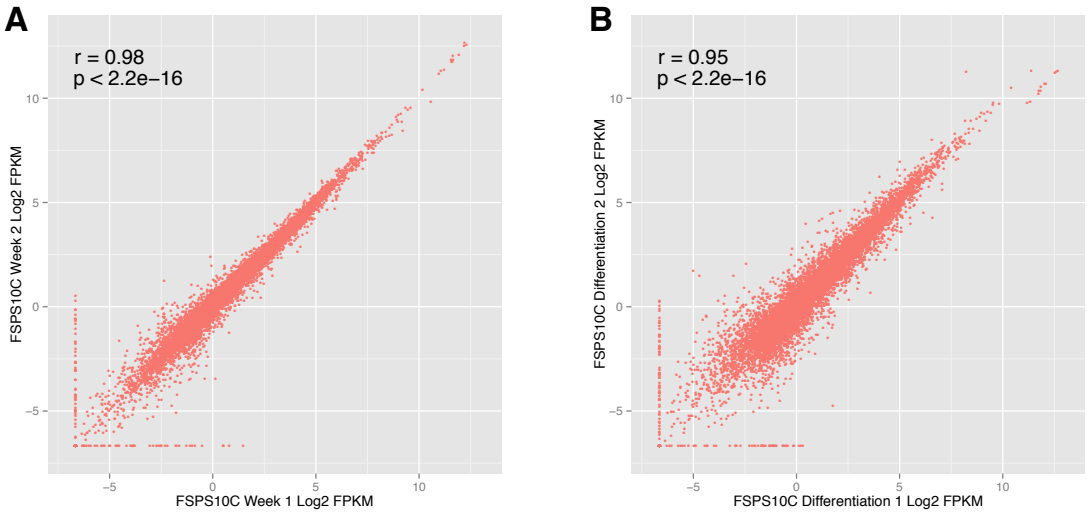
Supplementary Figure S5: Expression of five cell surface markers in macrophages derived from three independent IPS lines measured using flow cytometry. Blue indicates the population of cells stained for each surface marker. Solid and dotted lines correspond the unstained or isotype controls, respectively.



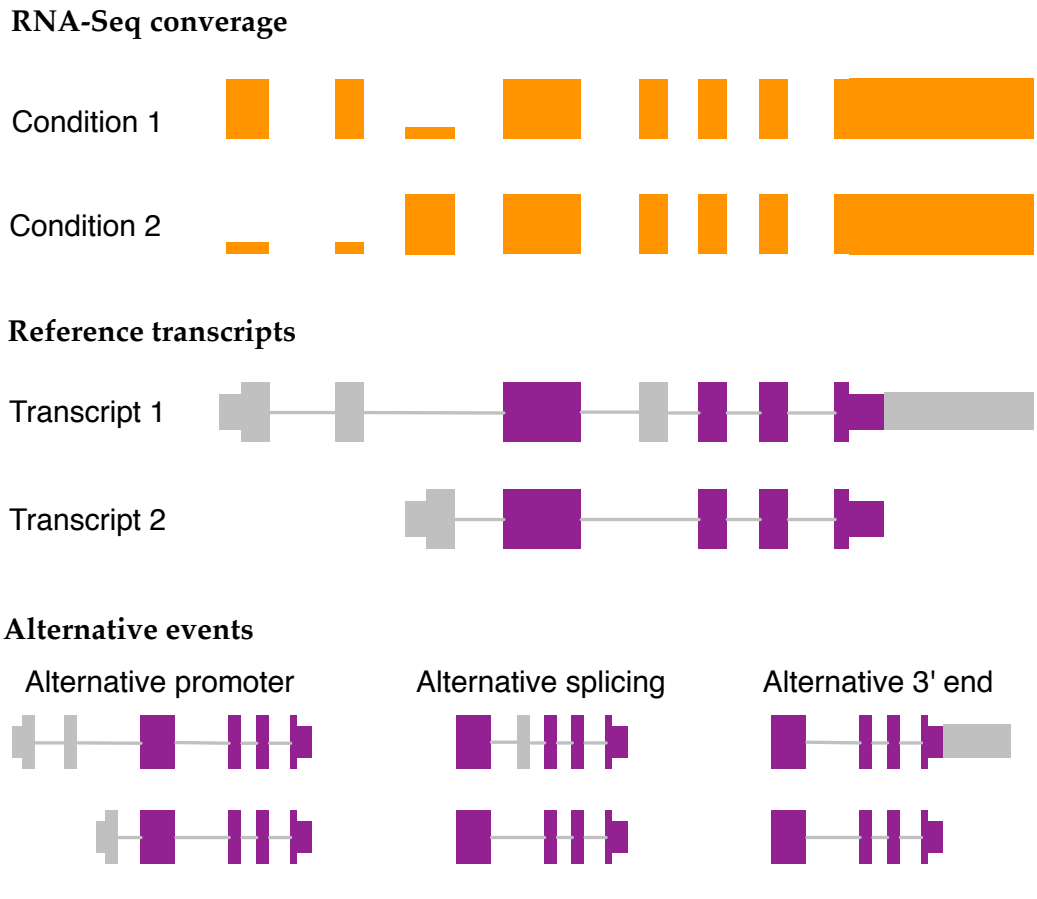
Supplementary Figure S6: (a) Expression levels of genes more highly expressed in IPSDMs compared to MDMs (TPM > 2). Purple violin plots show the mean expression of these genes in MDMs and orange in IPS cells. Red asterisks mark IPSDM samples (FSPS10C) that stained > 99% positive for CD14, CD206 and CD4 while S7RE and FSPS11B samples were ~91% positive.

(b) MA-plot of differentially expressed genes between MDMs and IPSDMs (without TPM cut-off). On the y-axis is the DESeq2 estimate of fold-change between MDMs and IPSDM. Red line denotes the 2 TPM cut-off used in most analyses. Most of the genes more highly expressed in MDMs have mean expression > 2 TPM. On the other hand, large proportion of genes more highly expressed in IPSDMs has extremely low mean expression level.

(c) Fraction of gene promoters overlapping H3K27Me3 peaks in ENCODE CD14+ monocyte samples stratified by the percentile of gene expression level. Genes upregulated in IPSDMs (red) were strongly enriched for repressive H3K27Me3 chromatin marks at their promoters compared to genes that were either downregulated (blue) or not differentially expressed (orange).



Supplementary Figure S7: Scatter plots of gene expression. (a) Scatter plot of expressed genes (FPKM > 0.3) in FSPS10C-derived macrophages harvested one week apart from the same differentiation culture. **(b)** FSPS10C macrophages harvested two months apart from independent differentiation cultures. Differentiation 2 also used recombinant M-CSF instead of supernatant from CRL10154 cell line.



Supplementary Figure S8: Constructing alternative transcription events from alternative transcripts. Top panel shows RNA-Seq coverage over a gene suggesting alternative promoter usage between conditions 1 and 2. Middle panel shows hypothetical transcripts detected to be differentially expressed in the same conditions. Due to inaccurate reference transcript annotations the second promoter is always linked to skipped exon 4 and short 3' UTR. Comparing these two transcripts to each other gives a wrong impression that exon 4 and 3' UTR are also differentially expressed. Lower panel shows alternative transcription events constructed from the two transcripts using reviseAnnotations. Estimating the expression of these alternative events separately correctly identifies that only the promoter usage changes between conditions.

Supplementary Table S1: Purity of iPS-derived macrophages. Percentage of cells expressing each of the five surface markers in stained and unstained populations measured by flow cytometry. Macrophages were differentiated from three different iPS cell lines (FSPS10C, FSPS11B and S7RE). Raw data is shown on Supplementary Fig. S5.

	FSPS10C		FSPS11B		S7RE	
Marker	unstained	stained	unstained	stained	unstained	stained
CD14	1.77	98.6	2.04	90.4	1.57	91.2
CD206	1.01	99.5	1.86	85.1		
CD4	1.91	99.5	1.96	92.8	2.09	92.9
CD32	2.14	94.8			2.12	87.6
CD163	1.91	74.1	2.02	92	2.09	85.6