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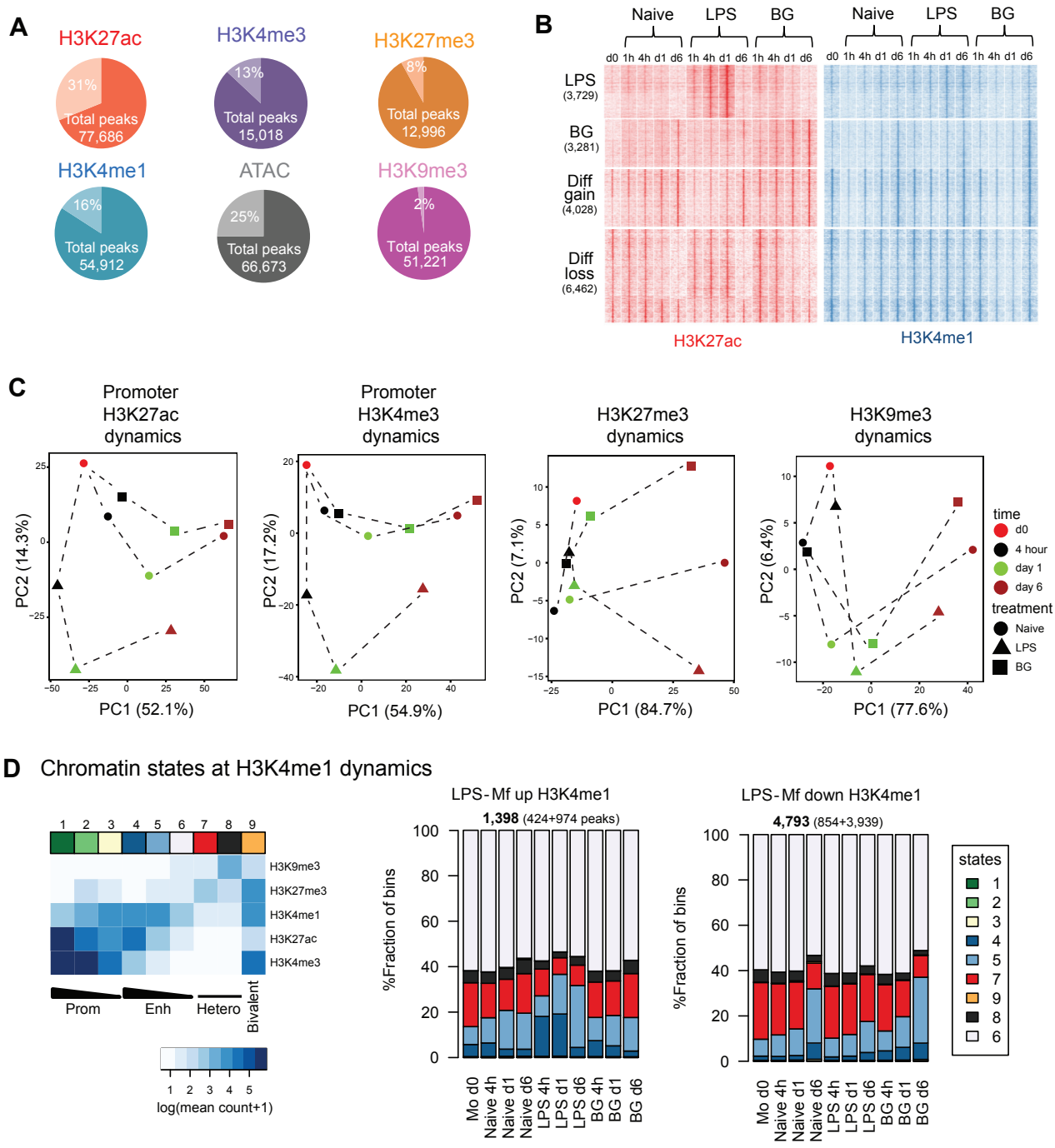


Figure S1. Summary of Dynamic Histone Marks and PCA Plots of Dynamic Active Histone Modifications at Promoters and Repressive Marks, Related to Figure 1

(A) Percentage of histone ChIP-seq peaks designated as dynamic across time-points and between treatments. H3K27ac was the most dynamic modification, with almost a third of regions showing significant changes.

(B) Heatmap showing histone intensity of H3K27ac and H3K4me1 at dynamic H3K27ac enhancers with 12kb ± from center of the peak.

(C) PCA plots for all time-points for H3K27ac dynamic promoters, H3K4me3 dynamic promoters, dynamic H3K27me3 regions, and dynamic H3K9me3 regions. H3K27ac and H3K4me3 at promoters behave similarly over time and in response to LPS or BG exposure, and reflect the behavior of H3K27ac at enhancers. Unlike active marks, repressive marks show little dynamics up to day 1.

(D) Stacked plots showing chromatin state changes over differentiation at "LPS-Mf up" and "BG up / LPS down" H3K4me1 enhancers. These enhancers are established through H3K27ac dynamics shown in Figure 1C. The genome was segmented into 9 chromatin states based on the 5 histone marks analyzed. This analysis indicates that H3K4me1 increase is associated with loss of H3K27me3.

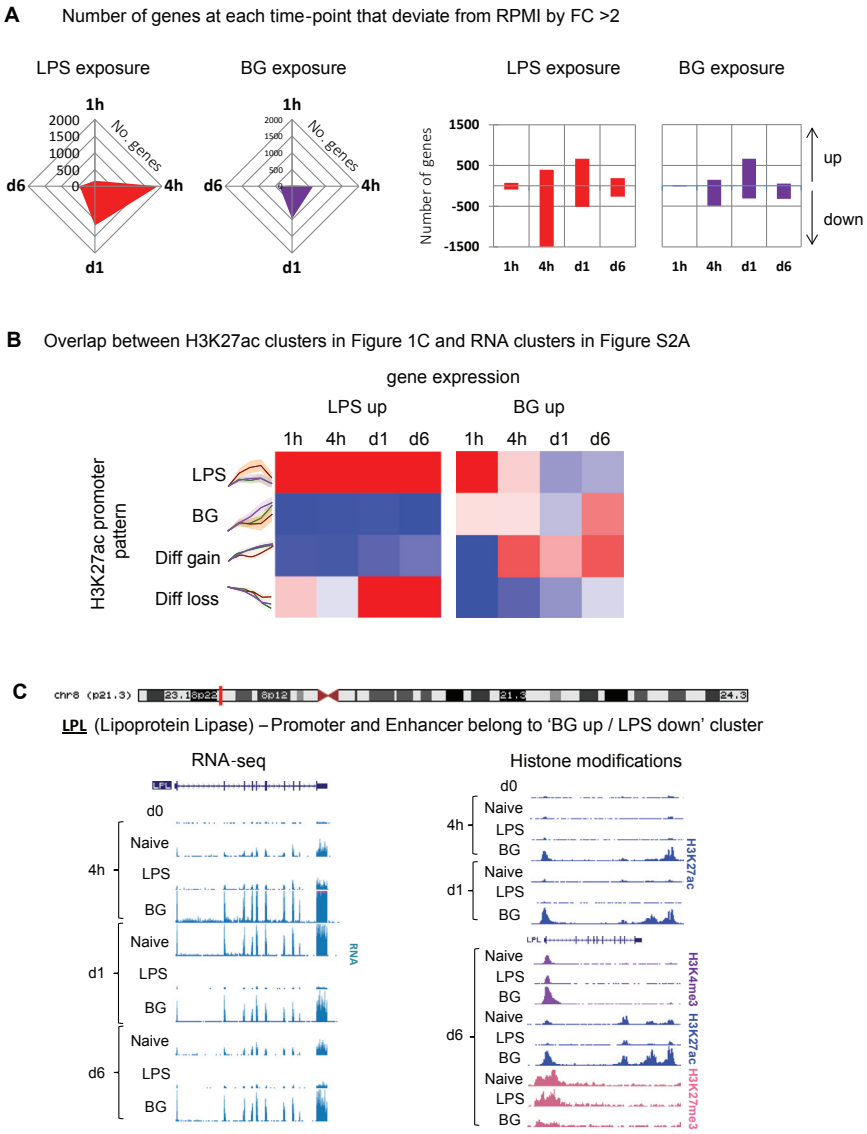


Figure S2. RNA-Seq Dynamics in Response to LPS and BG and Relationship to Histone Marks, Related to Figure 1

(A) Number of genes showing treatment (LPS or BG) specific expression at each time point (1h, 4h, d1, d6). LPS exposure induces the largest number of genes at each time-point, with a minimum of 110 transcripts at 1h, and a maximum of 650 transcripts at day 1. Up to 100 genes maintain LPS-specific expression at d6. Comparatively BG induced gene expression patterns peak at d1, a fraction of which is maintained to d6.

(B) Overlap between gene expression group and promoter H3K27ac cluster. LPS-induced H3K27ac accumulation at promoters correlates well with LPS induced gene expression at all time-points. However, at day 1 and day 6, the 'LPS-up' genes are equally explained by a lag in differentiation-associated repression in LPS treated cells. Conversely, BG exposure leads to faster expression of differentiation associated genes, with higher overlap between 'BG-up' genes and 'differentiation gain' and BG-associated H3K27ac promoters.

(C) Example tracks of a BG induced/LPS repressed gene and an LPS induced gene, LPL (Lipoprotein Lipase).

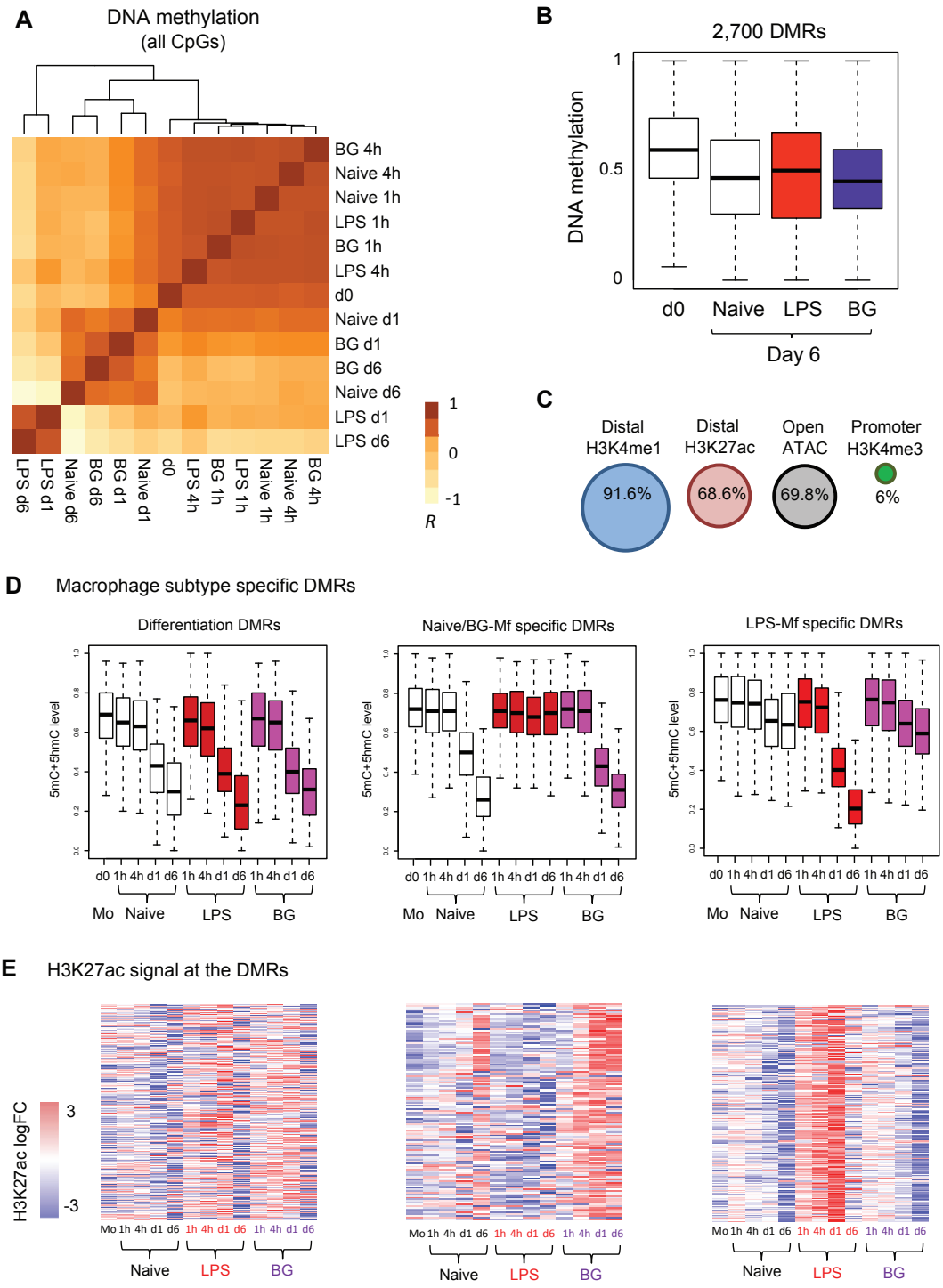


Figure S3. DNA Methylation Dynamics in Monocyte-to-Macrophage Differentiation and Tolerance and Training, Related to Figure 1

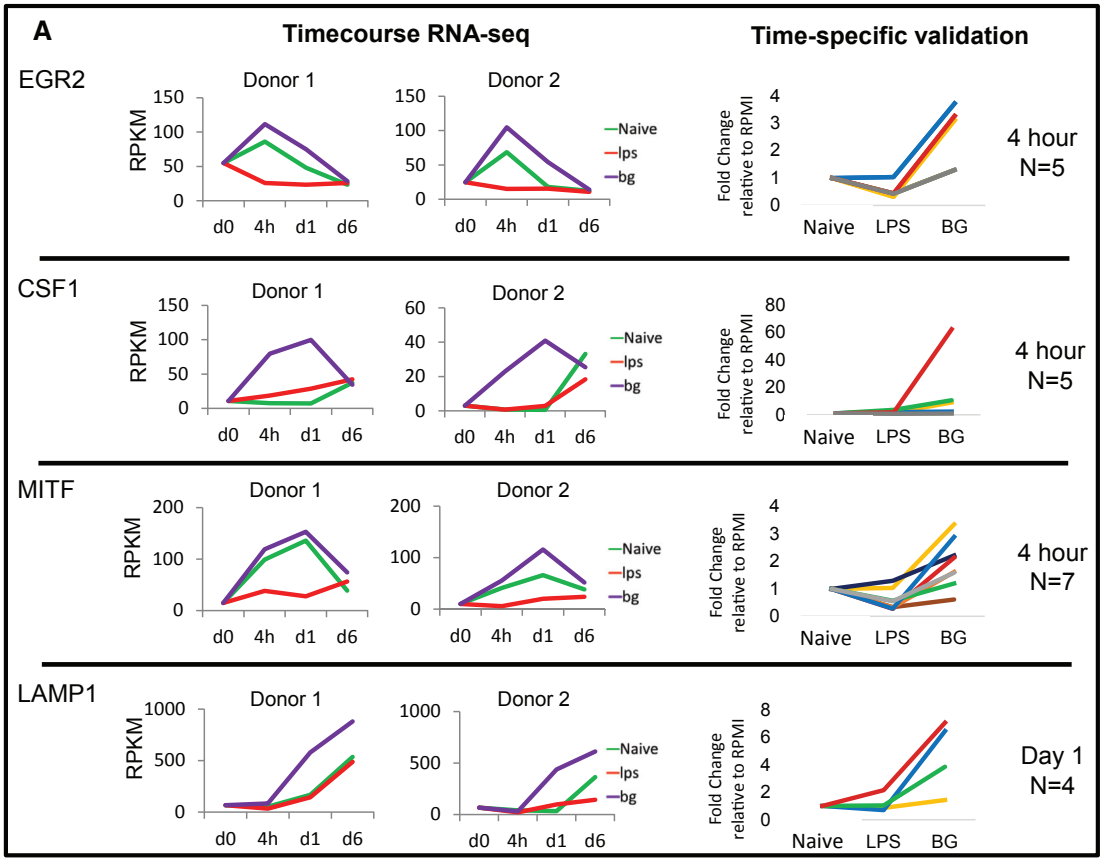
(A) Correlation plot of DNA methylation values, showing clear separation of LPS d1 and LPS-d6 from other samples.

(B) Boxplot of 2,700 DMRs, showing that the general trend is loss of methylation during monocyte-to-macrophage differentiation.

(C) Chromatin context of DMRs. The majority (91%) of DMRs occur in distal regions marked by H3K4me1, 69 occur at H3K27ac marked enhancers and open chromatin regions. Only 6% occur at promoters.

(D) Boxplots showing DNA methylation over time for macrophage sub-type specific DMRs. Analysis identified DMRs common to all macrophages, and those that are only established in LPS-Mf or not-established in LPS-Mf.

(E) Heatmap of H3K27ac changes at DMRs. Generally, DNA de-methylation at DMRs was associated with accumulation of H3K27ac.



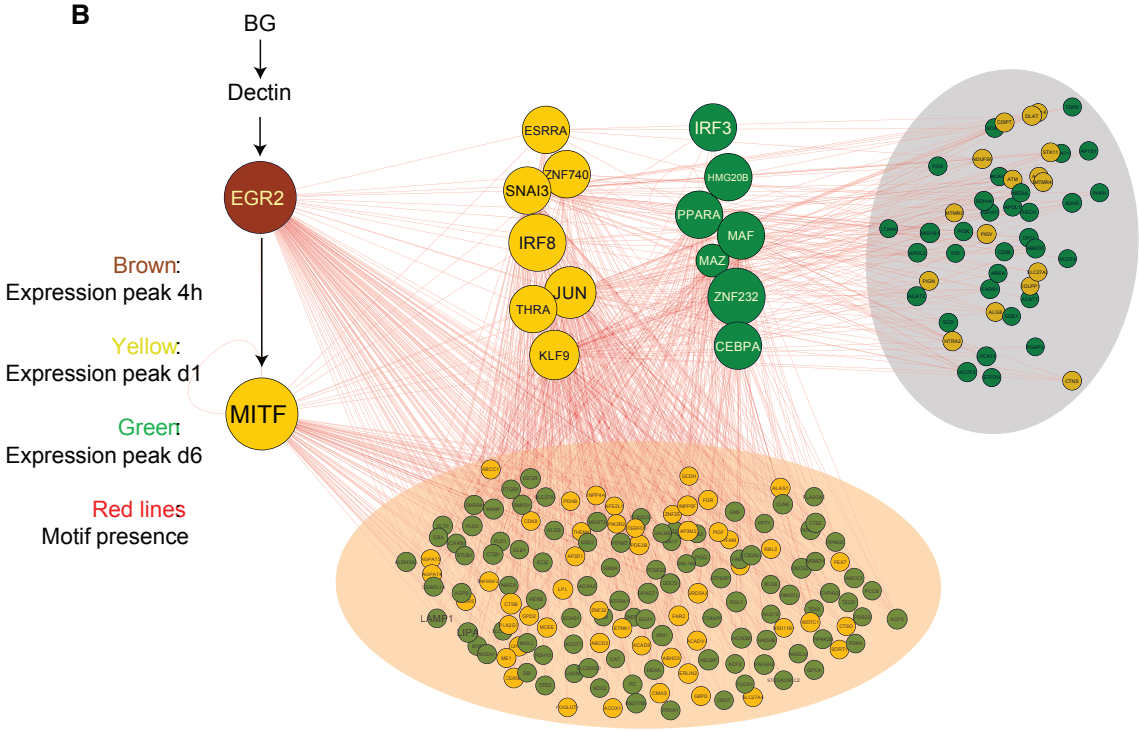


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Figure S4. Expression of Transcription Factors with Enriched Motifs at BG-Associated Promoters and Enhancers and Pathways Associated with Downstream Genes, Related to Figure 2

(A) The expression of main genes enriched at 'BG up / LPS down' and 'Differentiation gain' promoters and enhancers is shown separately for each donor over time. Naive cells are green, LPS exposed cells are red, and BG exposed cells are purple. *EGR2* expression peaks transiently at 4 hr in BG exposed cells, but by day 6, there is no difference between Naive, LPS-Mf or BG-Mf. *CSF1* and *MITF* expression peaks at day 1 and then is reduced. Downstream TF *USF2* shares one motif with *MITF*, and shows high expression in BG macrophages at day 6. *LAMP1* is a major component of the lysosome, and together with *LAMP2* makes up 50% of all lysosomal proteins. *LAMP1* expression peaks late, and is significantly higher in BG-Mf compared to naive and LPS-Mf. qPCR was used to validate RNA-seq results in monocytes from multiple donors.

(B) Transcription Factor network based on EGR2 and MITF motif occurrence at BG induced lysosome and lipid metabolism genes. The size of the nodes represents the number of connections. EGR2 motif is present in the MITF promoters (thick connection). EGR2 and/or MITF motifs are present in another 28 TFs, which themselves have 14 distinct motifs (and are visible as a cluster. Most genes have a combination of EGR2, MITF and a downstream TF motifs (light brown circle). The set of genes to the right do not have EGR2 or MITF motifs, but have motifs for one of the downstream TFs (light gray circle). Overall this network explains 79% of BG-induced lipid metabolism and lysosome-associated genes, compared to 58% based on EGR2 and MITF scan alone. BG induces *EGR2* expression, through its receptor, Dectin-1, and higher expression of *MITF* is observed, as well as its activator cytokine factor *CSF1* (see also Figure S4). Conversely, LPS treatment represses *EGR2*, *CSF1* and *MITF*. Genes are labeled by time at which their expression peaks in BG exposed cells. *EGR2* expression peaks at 4 hr (brown), *MITF* and *KLF9* at day 1 (gold). The rest of the downstream genes peak at day 1 (gold) or peak at day 6 (green). Connections between TFs and downstream genes is shown as red lines.

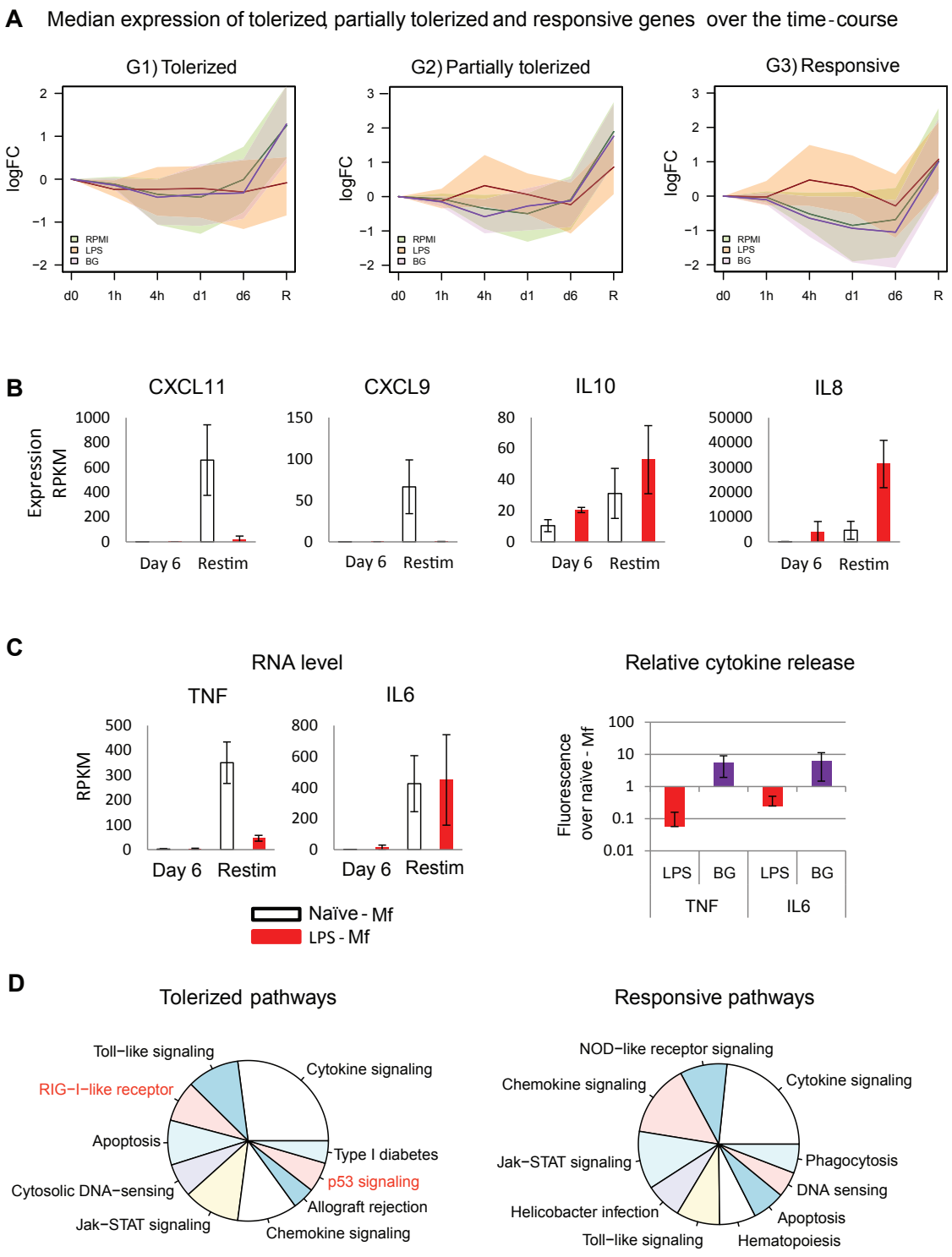

Figure S5. Tolerance at the Transcriptional Level, Related to Figure 3

(A) Pattern of expression of tolerized and responsive genes during the time-course shown as median logFC of two donors (with first and third quartiles shown as shaded areas). The most tolerized (G1) genes did not show upregulation in response to the initial LPS exposure in monocytes, while responsive genes (G3) showed high induction in monocytes.

(B) Notable examples of tolerized and responsive genes. Data are shown as mean RPKM and error bars are standard deviations. Data are represented as mean ± SD.

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(C) Expression of *IL6* and *TNF*. Release of these proteins from macrophages in response to LPS is considered the gold-standard for determining tolerance. At the transcriptional level TNF is partially tolerized, while IL6 is responsive in LPS-Mf. Error bars represent standard deviation. IL6 and TNF protein release after LPS restimulation is high in BG-Mf and absent in LPS-Mf compared to naive-Mf. The disconnect between transcription and release of IL6 can potentially be explained by the larger size and higher lysosome content in BG-Mf, induced by early activation of lipid and lysosome pathways in BG exposed cells.
(D) Top 10 KEGG pathways enriched in tolerized and responsive gene groups from DAVID ontology analysis. Area relates to the number of genes within the pathway, red font signifies that the pathway only shows significant enrichment in the tolerized gene group. Cytokine-cytokine receptor signaling was the top pathway in both tolerized and responsive groups indicating that cytokine genes are equally spread across the gradient of LPS-Mf response to LPS re-exposure.

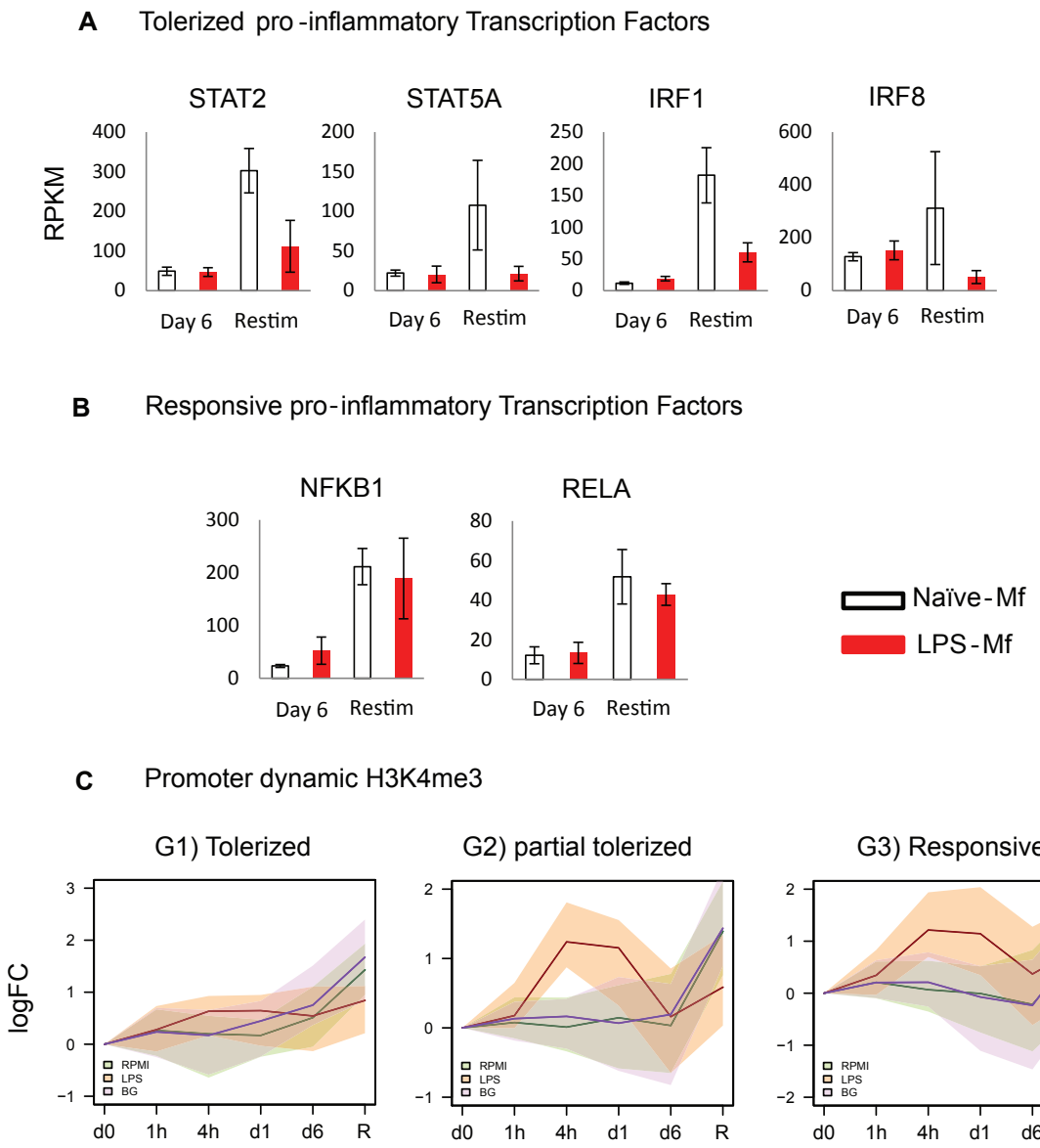

Figure S6. Active Histone Mark Changes at Promoters of Tolerized and Responsive Genes and Overall Chromatin States at the Same Promoters, Related to Figure 4

(A) Expression at day 6 and at LPS re-exposure for STAT2 and −5A, and IRF1 and −8 (mean RPKM of 4 donors, error bars represent standard deviation). These pro-inflammatory TFs show a tolerized response in LPS-Mf to LPS re-exposure. The inability of these genes to be activated may play a role in the tolerance of downstream targets, as suggested from the enrichment of their motifs in the G2 partially tolerized gene promoters (Figure 4B).

(B) expression at day 6 and at LPS re-exposure for NFKB1 and RELA. These TFs are responsive to LPS re-exposure in LPS-Mf, and their motifs are not significantly enriched in tolerized genes. This suggests that NF-κB signaling is not impaired at the level of transcription. Data are represented as mean ± SD.

(C) LPS-Mf do not accumulate H3K4me3 at tolerized genes, but do so at the promoters of responsive genes. This pattern is similar to that of H3K27ac shown in Figure 4D.

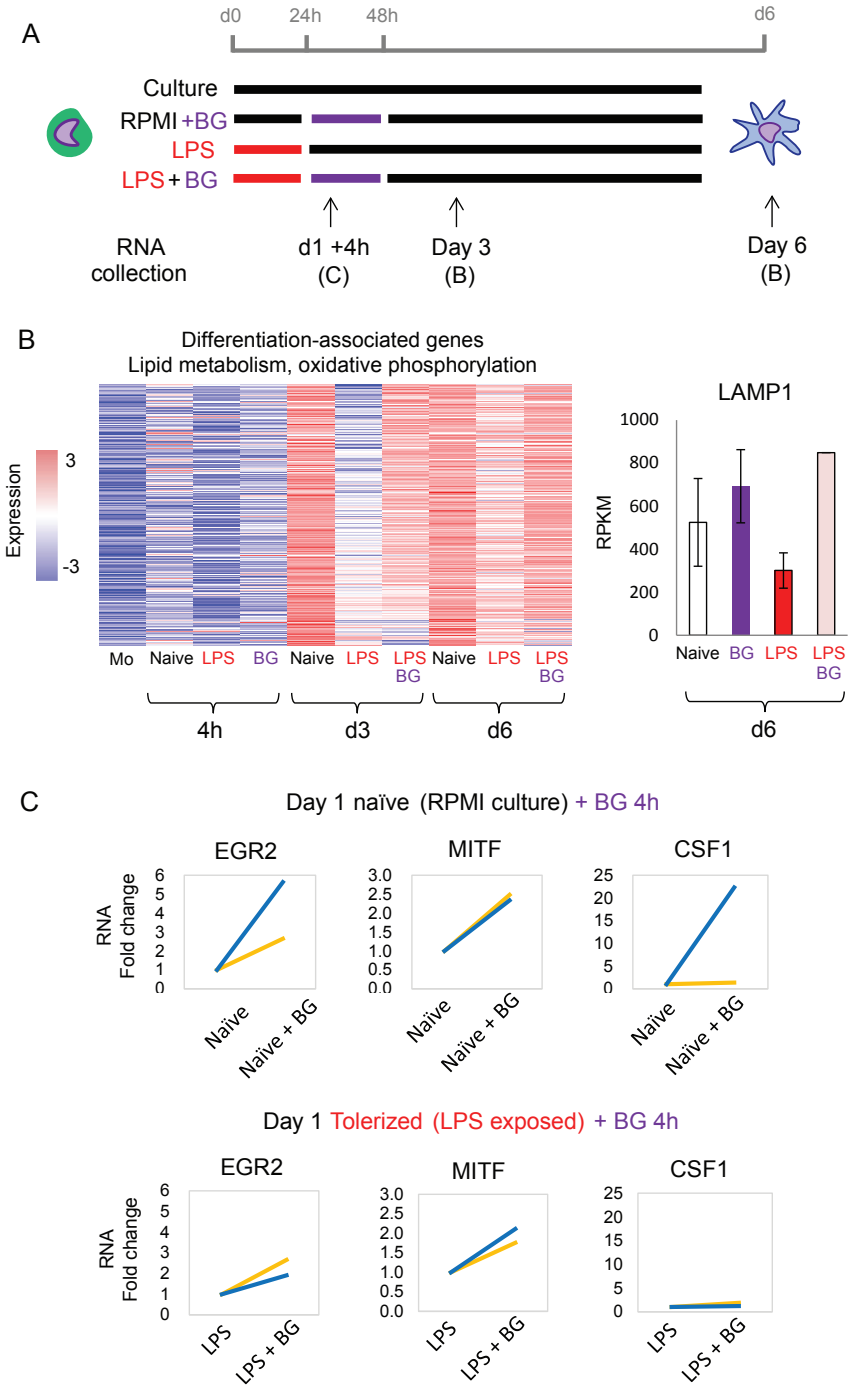

Figure S7. Expression of Genes Involved in Lipid Biosynthesis and Metabolism following BG Reversal of LPS-Induced Tolerance, Related to Figure 7

(A) Experimental set-up, indicating the collection of samples for gene expression analysis. Samples were collected at day 1 +4h, indicating that monocytes were treated with media (RPMI) or LPS for 24 hr, at which point cells were exposed to BG for 4 hr and collected. Additionally samples were collected at day 3 and day6.

(B) BG exposure, following LPS, recovers the expression of genes involved in lipid biosynthesis and oxidative phosphorylation as early as day 3. LAMP1 is an example of a lysosome gene that shows high expression in BG-Mf and low expression in LPS-Mf. BG exposure recovers the expression of this gene in LPS-BG-Mf.

(C) BG addition at day 1 in Naive monocytes induces the expression of EGR2, MITF and CSF1, as it does when added at day 0 (Figure S4C). In tolerized monocytes, BG induces the expression of EGR2 and MITF, but to a lesser degree. This indicates that BG receptor pathways are not completely disrupted by LPS exposure, providing a basis for BG reversal of LPS-induced tolerance.