Supporting Information

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SI Materials and Methods

Detection of Secreted Cytokines, Surface Antigens, and Intracellular Proteins. The following cytokines were detected with ELISA using commercially available antibody pairs or kits: mouse IL-6 (BD Bioscience), IL-1α (eBioscience), IL-1β (eBioscience), IL-10 (eBioscience), human IL-6 (R&D Systems), IL-1α (BioLegend), and IL-10 (BD Bioscience). IFN-αβ and TNF-α activities were measured bioassays as described (1). Surface markers were detected with FACS using the following antibodies: major histocompatibility class II I-Ab (AF6-120.1), granulocyte differentiation antigen-1 (RB6-8C5), and cluster of differentiation molecule (CD)11b (M1/70) from BD Pharmingen; mouse homolog of EGF-like module-containing mucin-like hormone receptor-like (F4/80, BM8) and CD11c (N418) from eBioscience; CD32/16 (93) and CD14 (Sa14-2) from BioLegend; and macrophage receptor with collagenous structure (ED31) from AbD Serotec. Intracellular proteins were detected on immunoblots with antibodies from Pharmingen BD (IL-1α and IL-1β) and Cell Signaling (phosphorylated isophorm of p38 MAPK).

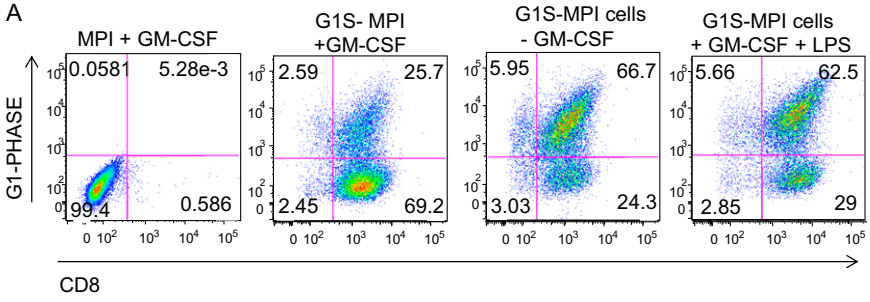
Microarray and Cluster Analysis. All microarrays were preprocessed and quantile-normalized with the RMA package (2). For the unsupervised cluster analysis, we calculated the Spearman correlation coefficient, C, for all pairs of probesets and defined a corresponding distance measures as (1 − C)/2. Based on those distances we performed a hierarchical clustering using the function hclust in R, which give rise to the dendrograms of Fig. 2. To highlight functional differences between the different cell types, the global cluster analysis for all genes was complemented by an identical analysis for targeted subsets of 285 genes associated with cell-cycle processes [Gene Ontology (GO) 0007049] and 456 immune response genes (GO 0006955).

Linear Modeling. To account for the systematic variability in the data, we applied a linear model for each gene and determined a number of statistics, such as fold changes, *t*-statistics, and *P* values for the contrasts between specified conditions (different treatments/cell types). An empirical Bayes method was applied to moderate the *t*-statistics using the information from all genes. For all of the above steps, we used the functionalities provided by the limma-package in R (3). A false discovery rate (FDR) threshold of 5% was chosen to define subsets of differentially expressed genes. To define differential genes upon LPS treatment in different cell types, we require that a probeset is significantly up-regulated in one but not the other cell type [generated from BM precursors with M-CSF (BMM), Max Planck Institute (MPI)] and that the cell effect is also significant at a 5% FDR.

Enrichment Analysis. To identify functional differences more systematically, we used the gene set enrichment analysis (GSEA) on preranked gene lists. The ranking for different contrasts was based on the moderated *t*-statistics from limma, and we investigated possible enrichment in gene ontologies and canonical pathways from the MSigDB database (4). Importantly, this method does not impose any cutoff on the rank list of genes, but instead uses a model of random rank lists to assess the significance of observed deviations for any given functional category. Because many categories were being tested, multiple testing corrections were applied to rank the categories by their FDR.

1. Fejer G, et al. (2008) Key role of splenic myeloid DCs in the IFN-alphabeta response to adenoviruses in vivo. *PLoS Pathog* 4(11):e1000208.
2. Irizarry RA, et al. (2003) Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* 31(4):e15.
3. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3:Article3.
4. Subramanian A, et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102(43):15545–15550.

A



B

Week	3LL	MPI sc	MPI ip
1	3/4	4/4	4/4
2	0/4	4/4	4/4
12	-	4/4	4/4

Fig. S1. MPI cells are growth factor-dependent nontransformed cells. LPS treatment and GM-CSF deprivation arrests replicating MPI cells in the G1 phase of cell cycle. MPI cells transduced with a retrovirus coding for CD8 and a reporter protein (G1S-MPI) emitting at 578 nm in G1 phase only were analyzed when grown with GM-CSF, with GM-CSF in addition to 0.1 μg/mL LPS for 16 h, or for 2 d in the absence of GM-CSF. Control nontransduced, GM-CSF cultured MPI cells are shown in *A*, *Lower Left*. MPI cells are not tumorigenic. Recombination-activating gene-2$^{-/-}$ mice (four per group) were injected with 2× 10^6 Lewis lung carcinoma cells (3LL) i.p. or with MPI cells s.c. or i.p. The number of living, tumor-free animals at the indicated time points after injection is shown (*B*).

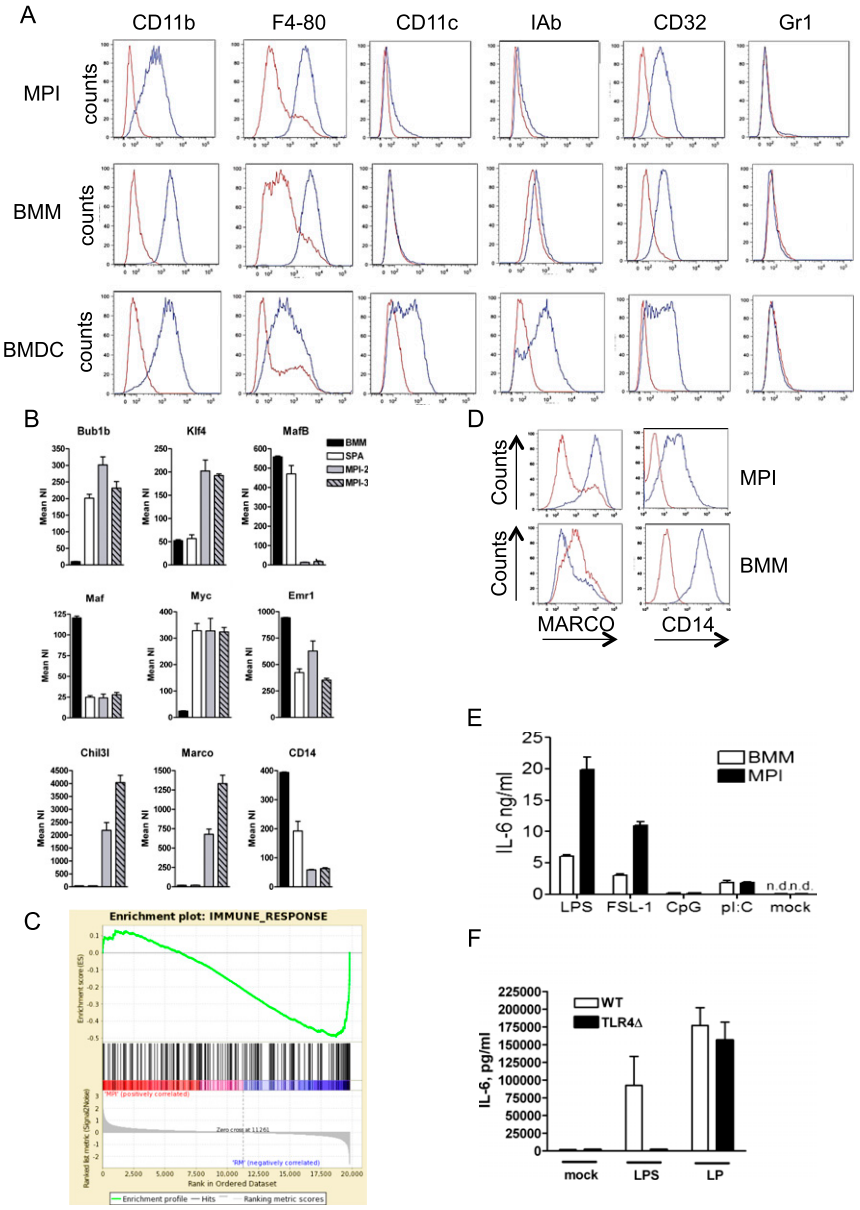


Fig. S2. Gene expression profiles and innate responses in MPI cells. (*A*) FACS staining of MPI cells, BMMs, and BM-derived dendritic cells (BMDCs) with isotype (red) or specific antibodies (blue). Expression levels of selected genes in BMMs, SP37A3, MPI-2, and MPI-3 cells. (*B*) Normalized intensity values (NI) ($n = 3$) from the microarray data. Gene set enrichment analysis and innate responses in MPI cells. Enrichment plot of the "immune response" pathway shown as an example of a differentially regulated pathway in MPI cells. First genes are ranked according to their differential expression score (red, up-regulated in MPI vs. BMM; blue, down-regulated in MPI vs. BMM). Then each pathway is scored by the rank distribution of its genes and statistically evaluated by the GSEA algorithm (*C*). Expression of MARCO and mCD14 on MPI cells and BMMs detected by FACS using specific (blue) or isotype control antibodies (red) (*D*). IL-6 levels in cell-free supernatants of MPI cells and BMMs stimulated with 0.1 μg/mL S-LPS, 0.1 μg/mL fibroblast stimulated lipopeptide-1 (FSL-1), 8 nM CpG ODN 1668, or 25 μg/mL polyriboinosinic polycytidylic acid (*E*). IL-6 levels secreted by WT and toll-like receptor(TLR)4-deficient (ΔTLR4) MPI cells in response to 0.1 μg/mL LPS or 0.1 μg/mL lipopeptide FSL-1 (LP) (*F*).

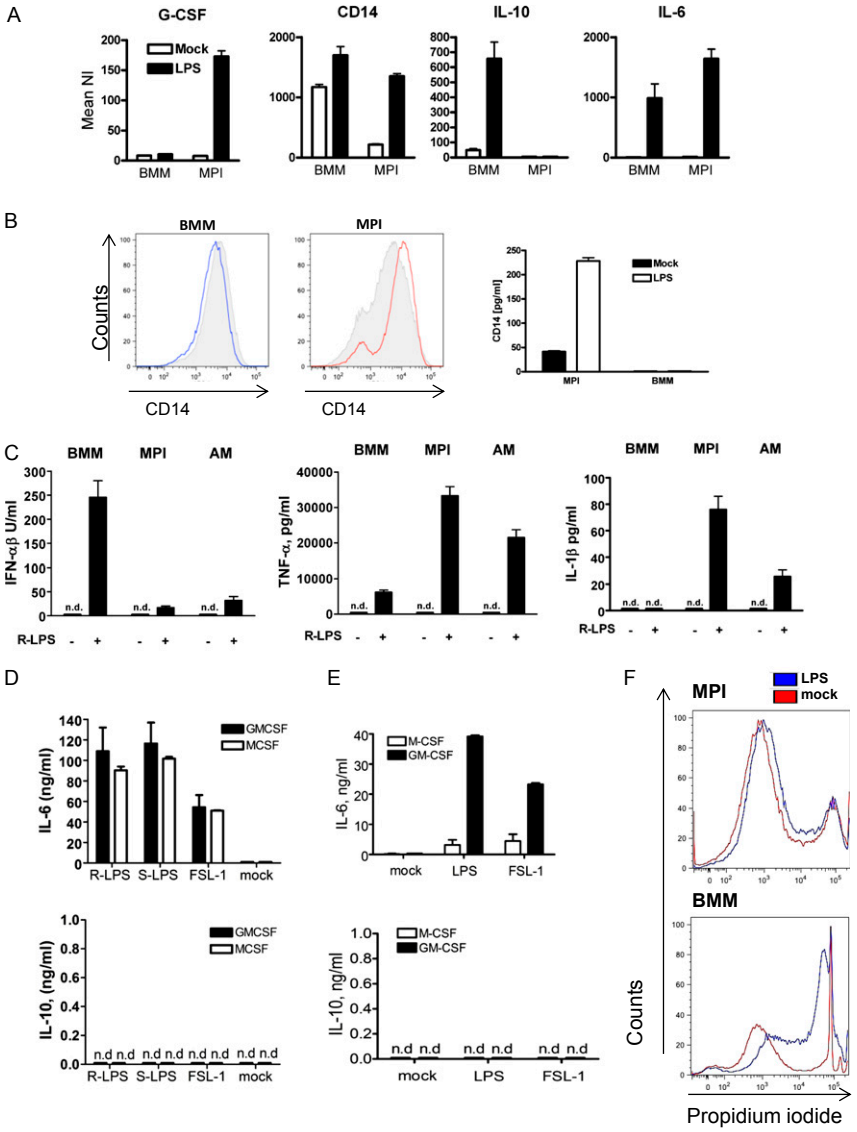


Fig. S3. Differences between LPS-stimulated responses of MPI cells and BMMs. Induction of selected genes in BMMs and MPI-2 cells induced by 0.1 μg/mL LPS. (*A*) NI values ($n = 3$) from the microarray data. (*B*) Induction of CD14 in MPI cells in response to LPS: MPI cells and BMMs were stimulated (blue and red line, respectively) or not (grey area) with 0.1 μg/mL LPS for 16 h, and membrane-bound CD14 was measured by FACS (*Left* and *Center*) and soluble CD14 in cell-free supernatants by ELISA (*Right*). Induction of IFN-αβ, TNF-α, and IL-1β in supernatants of BMMs and MPI cells stimulated with 0.1 μg/mL LPS (*C*). IL-6 responses of MPI cells stimulated in the presence of GM-CSF or M-CSF with 0.1 μg/mL LPS and 0.1 μg/mL FSL-1 (*D*). IL-6 responses of MPI cells grown with GM-CSF or after substitution of GM-CSF for 3 wk with M-CSF to 0.1 μg/mL LPS, 0.1 μg/mL FSL-1 (*E*).

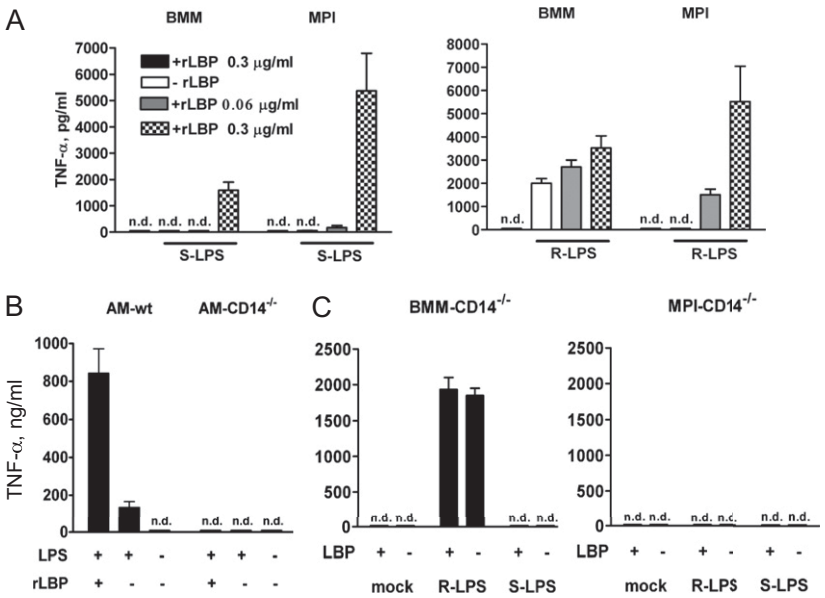


Fig. S4. MPI cells and AMs require CD14 to sense rough LPS. TNF-α response of BMMs and MPI cells to smooth form LPS (S-LPS) or rough form LPS (R-LPS) (0.1 μg/mL) in serum-free medium with or without recombinant LBP (*A*). TNF-α response of AMs from WT and $CD14^{-/-}$ mice to 0.01 μg/mL R-LPS with or without recombinant LPS binding protein (rLBP) (*B*). TNF-α response of $CD14^{-/-}$ BMMs and MPI cells to 0.1 μg/mL R-LPS or S-LPS with 5% of serum of WT or $LBP^{-/-}$ mice (*C*).

Dataset S1. Results from a functional enrichment analysis when comparing MPI cells with BMM cells using microarray data of triplicate samples of total cellular RNA

Dataset S1

The first column contains the name of the enriched pathway as used by MsigDB and the second column denotes the FDR of the observed enrichment as obtained from the GSEA algorithm (1). Only enrichments with an FDR smaller than 5% are shown.

1. Subramanian A, et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102(43): 15545–15550.

Dataset S2. Results from a functional enrichment analysis when comparing BMM cells with MPI cells using microarray data of triplicate samples of total cellular RNA

Dataset S2

The first column contains the name of the enriched pathway as used by MsigDB and the second column denotes the FDR of the observed enrichment as obtained from the GSEA algorithm (1). Only enrichments with an FDR smaller than 5% are shown.

1. Subramanian A, et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102(43): 15545–15550.

Dataset S3. Results from a functional enrichment analysis when comparing SP37A3 dendritic cells with MPI cells using microarray data of triplicate samples of total cellular RNA

Dataset S3

The first column contains the name of the enriched pathway as used by MsigDB and the second column denotes the FDR of the observed enrichment as obtained from the GSEA algorithm (1). Only enrichments with an FDR smaller than 5% are shown.

1. Subramanian A, et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102(43): 15545–15550.

Dataset S4. LPS-induced pathways in MPI cells

Dataset S4

The format of the dataset is as in Datasets S1–S3. Microarray data using newly synthesized RNA 60–90 min after stimulation with 0.1 μg/mL LPS. The enrichment analysis was performed with respect to mock-treated controls.

Dataset S5. LPS-induced pathways in BMMs

Dataset S5

The format of the dataset is as in Datasets S1–S3. Microarray data using newly synthesized RNA 60–90 min after stimulation with 0.1 μg/mL LPS. The enrichment analysis was performed with respect to mock-treated controls.

Dataset S6. Exclusively activated genes in MPI cells in response to LPS

Dataset S6

This dataset contains the list of probesets that are up-regulated upon LPS induction exclusively in MPI cells. Microarray data using newly synthesized RNA from 60 to 90 min after stimulation with 0.1 μg/mL LPS. The table columns contain the output of the limma package, which includes the probeset ID, the logtwofold-change, moderated *t*-statistics, *P* value, adjusted *P* value, and the *B*-statistics. For reference we have added the corresponding gene names and the normalized mean intensities of triplicate samples from BMM and MPI cells. Values for LPS induction (*_LPS) and mock-treated controls (*_C) are shown.

Dataset S7. Exclusively activated genes in BMMs in response to LPS

Dataset S7

This dataset contains the list of probesets that are up-regulated upon LPS induction exclusively in BMM cells. Microarray data using newly synthesized RNA from 60 to 90 min after stimulation with 0.1 μg/mL LPS. The table columns contain the output of the limma package, which includes the probeset ID, the logtwofold-change, moderated *t*-statistics, *P* value, adjusted *P* value, and the *B*-statistics. For reference we have added the corresponding gene names and the normalized mean intensities of triplicate samples from BMM and MPI cells. Values for LPS induction (*_LPS) and mock-treated controls (*_C) are shown.