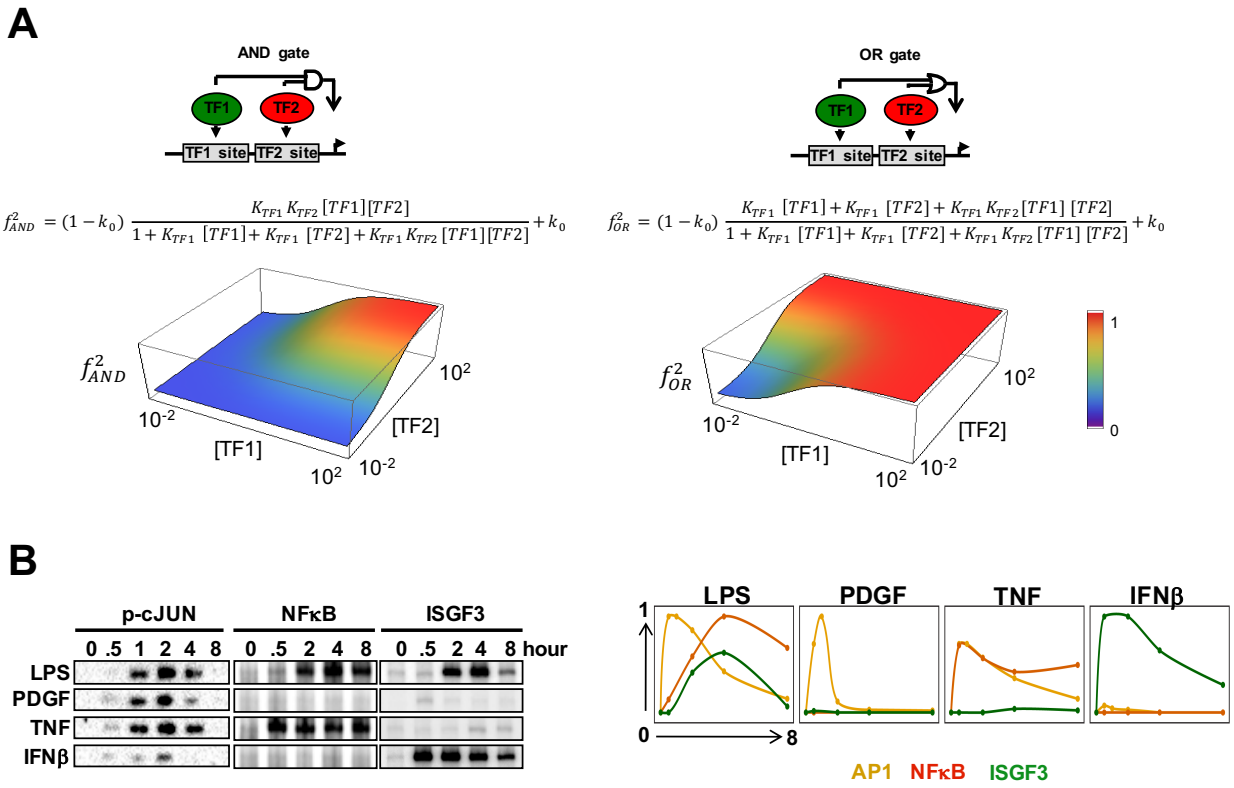

Figure S1, related to Figure 2. Modeling promoter activity as a function of pathogen-responsive transcription factors.

(A) Thermodynamic expressions of AND and OR logic gates.
Schematic of the Boolean logic gates and the mathematical expressions derived by Bintu et al 2005. The heatmaps show the behavior of each as a function of TF1 and TF2 activities.

(B) Pathogen-responsive TFs AP1, NFκB and IRF are induced by cytokines and growth factors.
Extracts prepared from MEFs at indicated times following treatment with LPS (0.1 μg/ml), PDGF (50 ng/ml), TNF (10 ng/ml), IFNβ (500 units/ml) were used for biochemical analysis to measure TF activities. AP1 activity was revealed by p-cJun immunoblot of whole cell tracts. NFκB and IRF DNA binding activities were revealed by EMSA with nuclear extracts. The shown data is representative of three independent experiments. On right, quantified experimental data, normalized to maximum, shown for each stimulation condition; this was used as input for model simulations.

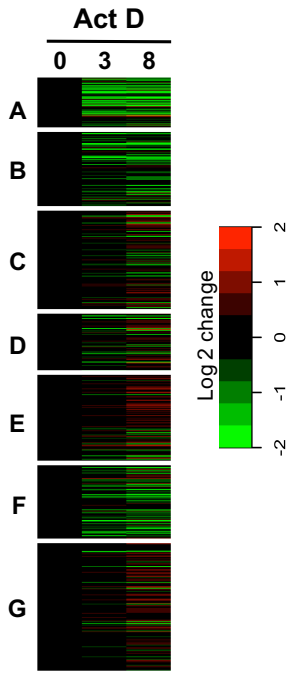

Figure S2, related for Figure 3. Actinomycin D treatment reveals differential mRNA half-lives in gene expression clusters.
Heatmap of mRNA abundances measured by Illumina bead array in MEFs following treatment with the transcription inhibitor Actinomycin D. Data from 714 genes grouped into 7 clusters (defined in Figure 2D) is shown. Further analysis of this data is shown in Figure 3B.

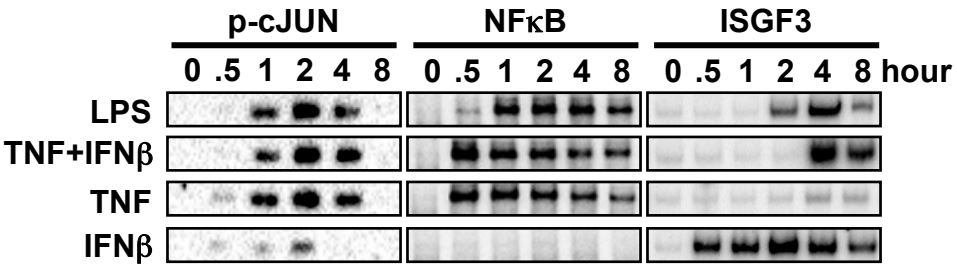

Figure S3, related to Figure 4. TNF and IFNβ co-stimulation results in TF activation profiles that are similar to those induced by LPS.

Extracts were made from MEFs at indicated times following treatment with LPS (0.1 μg/ml), TNF (10 ng/ml), IFNβ (500 units/ml) or the combination treatment of TNF and IFNβ at 2 hrs. AP1 activity was revealed by p-cJun immunoblot of whole cell tracts. NFκB and IRF DNA binding activities were revealed by EMSA with nuclear extracts. The shown data is representative of three independent experiments.

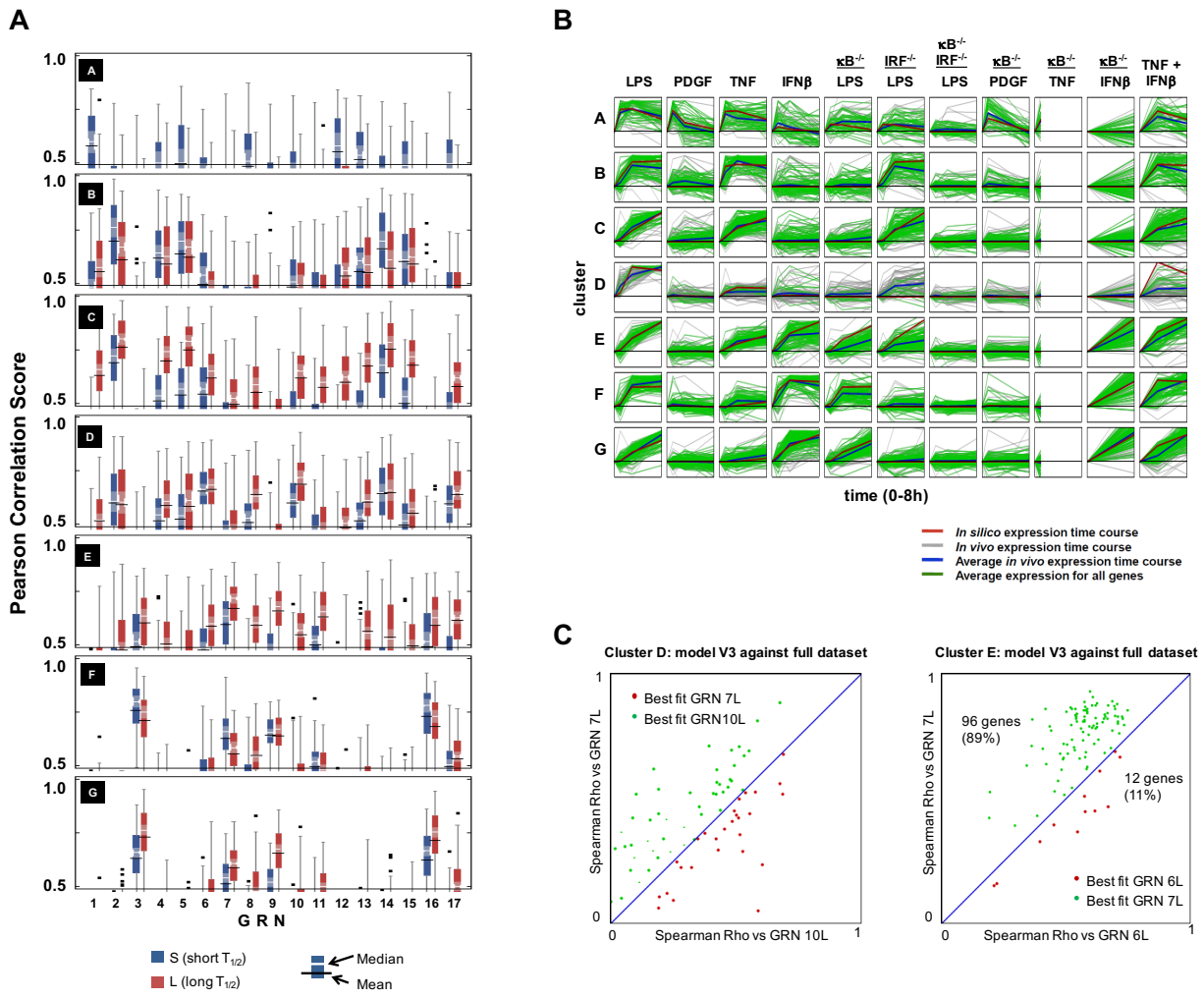

Figure S4, related to Figure 4. Performance of GRN models at the individual gene level.
(A) Testing GRN model assignments at the individual gene level. Pearson Correlation Score of observed MEF mRNA profiles for individual genes in each cluster and the predicted mRNA profiles for each of the 17 GRNs, each of which is shown with a short or long mRNA half-life (blue and red, respectively). This analysis includes all datasets presented in Figures 2, 3 and 4. Mean and median are indicated; boxes comprise 25-75% of the data, whiskers indicate 5% and 95%, and outliers are indicated. The analysis indicates that the best performing models are GRN1S for cluster A, GRN2S for cluster B, GRN2L for cluster C, GRN10L for cluster D, GRN7L for cluster E, GRN3S for cluster F, and GRN3L for cluster G, thus validating the original GRN model assignment in Figure 2H. However, the actual correlation score for assigned GRN models and how much better they are than for unassigned GRN models differ between clusters, indicating limits to model identifiability and potential intra-cluster heterogeneity.
(B) Spearman rank correlation statistics reveal that the GRN model assigned to cluster D performs more poorly than the GRN models assigned to the other clusters. Line graphs of MEF mRNA time course data for individual genes within each cluster. Those that passed (green) or did not pass (grey) goodness of fit criteria based on Spearman correlation statistics are distinguished by color. Experimental cluster average expression (blue) and GRN model predictions (red) are indicated also.
(C) Spearman correlation statistics confirm the distinction between AND and OR gate GRN models. Scatterplots of Spearman rank correlation coefficient Rho calculated for the expression profiles of each gene within cluster D (left) and cluster E (right), with respect to predicted mRNA profiles of GRN models 7L (OR gate) versus 10L/6L (AND gate). The scatterplot reveals that the OR gate GRN7L does not perform better than the assigned AND gate GRN10L for cluster D, and that the AND gate GRN6L does not perform better than the assigned OR gate GRN7L for cluster E.

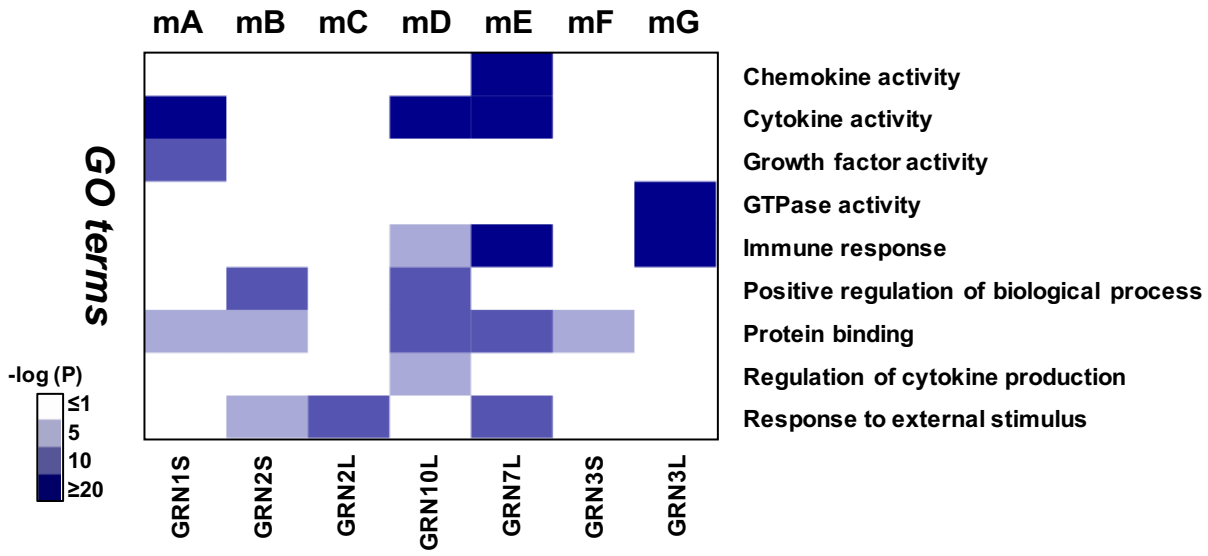

Figure S5, related to Figure 5. Gene Ontology analysis of macrophage expression clusters. Clusters of co-regulated genes identified in Figure 5 showed differential physiological functions as summarized by gene ontology analysis.

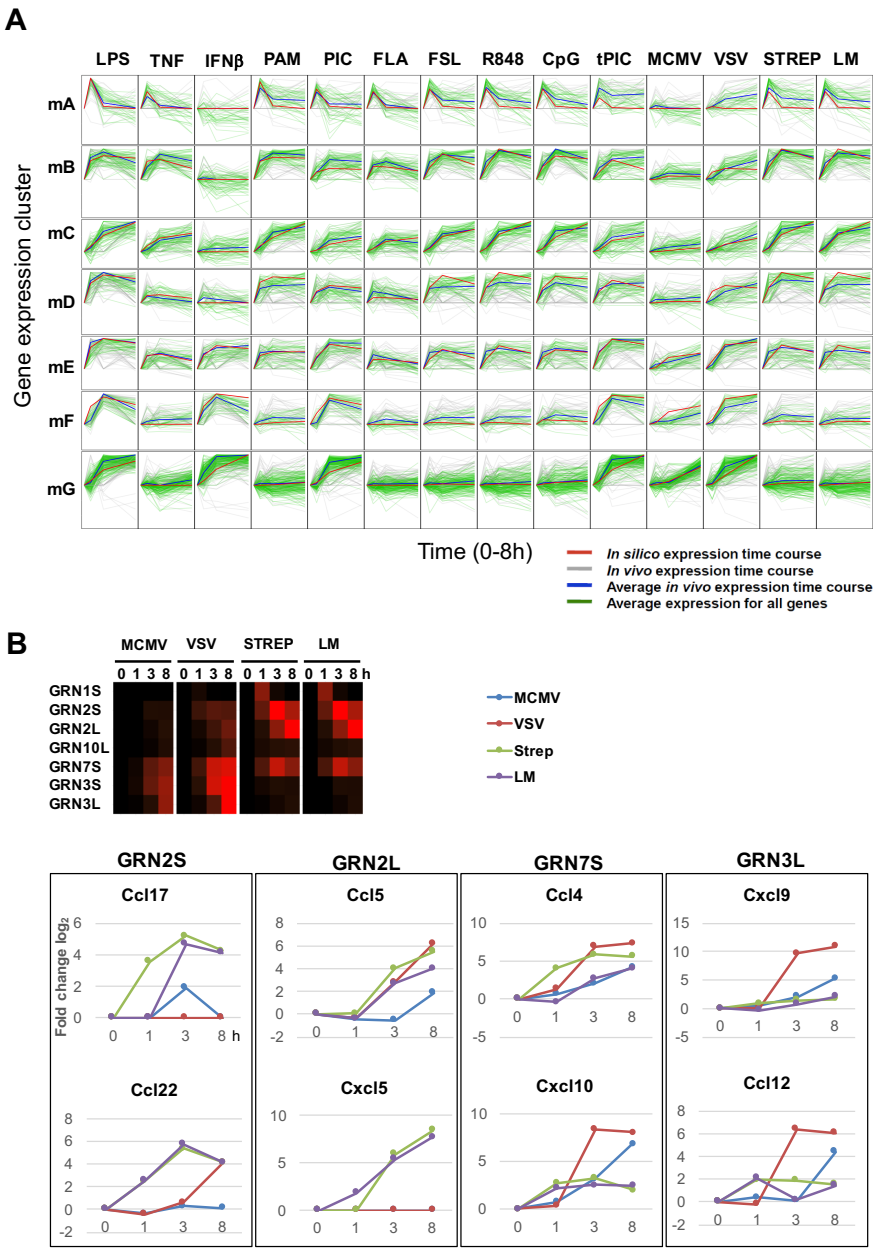

Figure S6, related to Figure 5. Model predictions match the majority of macrophage PAMP- and pathogen-responsive transcriptome measurements.

(A) BMDM mRNA profiles of individual genes that passed (green) or did not pass (grey) goodness of fit criteria based on Spearman rank correlation statistics are shown. Experimental gene expression cluster averages (blue) and model prediction (red) are indicated. This analysis is analogous to that shown in figure S4B.

(B) Predictions of pathogen-specific gene expression prove accurate when examined for a set of immune regulatory chemokine genes. Top left, heatmap of model-predicted gene expression patterns in response to indicated viral and bacterial pathogens (MCMV = Murine cytomegalovirus; VSV = vesicular stomatitis virus; STREP = *Strepococcus pneumoniae*; LM = *Listeria monocytogenes*). Bottom panels, experimental expression patterns for two example chemokine genes for each indicated GRN. GN2S and GRN2L are primarily induced in response to bacterial pathogens as they are NFκB-responsive. GRN7S is induced to all pathogens though strongest to VSV, as it is an OR gate of NFκB and IRF. GRN3L is induced to viral pathogens as it is responsive to IRF.

A

cluster	A	B	C	D	E	F	G		
model versions								additional perturbations	total data#
v.1 kinetic	45%	60%	62%	46%	75%	75%	65%	defined stimuli	12
	14%	77%	76%	10%	85%	92%	82%	knockouts	25
v.2 PDGF loop	71%	77%	76%	10%	85%	92%	82%		25
	73%	80%	80%	17%	87%	92%	86%	mixed stimuli	27
v.3 p38 axis	73%	80%	80%	73%	87%	92%	86%		27

B

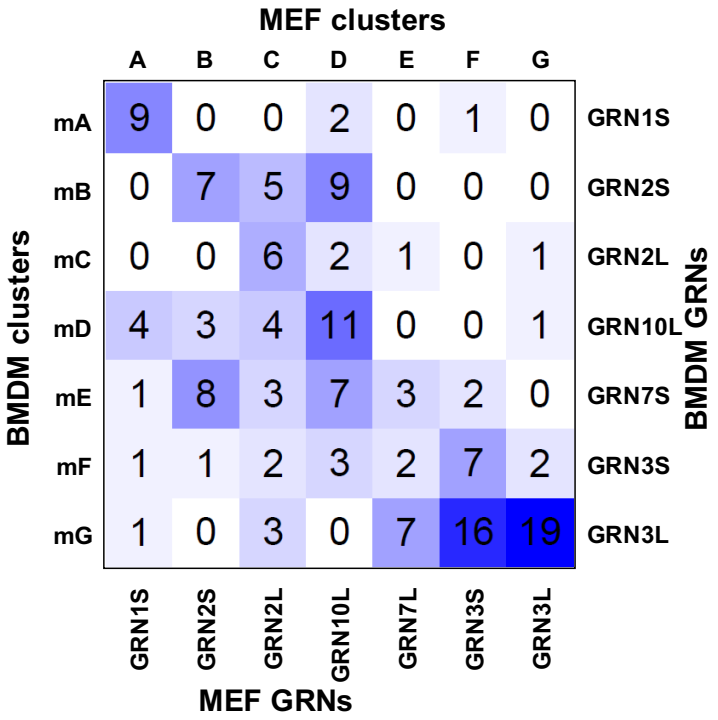

Figure S7, related to Figure 7. Scoring model performance and comparing regulatory logic control in two cell types.
(A) Fraction of genes passing the "goodness of fit" test for each MEF model version. Nature and number of datasets are indicated on the two rightmost columns. Relate to Figure 7A and Methods.
(B) Cross-comparison of those genes that are inducibly expressed in both MEFs and BMDMs. Numbers of genes are indicated that are assigned to specific GRN in each cell type. Most genes fall into the same GRN in each cell type, but some do not, indicating differential regulatory control logic.

Table S1, related to Figure 2. TF Input profiles for MEF GRNs

Table of nuclear TF activities, determined by quantifying immunoblots (p-cJun) and EMSAs (NFκB and ISF3), and normalizing basal and maximum activity values to 0.05 and 1, respectively.

A) AP1 activation profile

	0 min	**30 min**	**60 min**	**120 min**	**240 min**	**480 min**
LPS	0.05	1.	1.	0.864286	0.457143	0.185714
TNF	0.05	0.728571	0.728571	0.592857	0.389286	0.185714
PDGF	0.05	0.728571	1.	0.158571	0.0771429	0.069
IFN	0.05	0.117857	0.0907143	0.0771429	0.05	0.05

B) NFκB activation profile

	0 min	**30 min**	**120 min**	**240 min**	**480 min**
LPS	0.05	0.178824	0.604633	1.	0.688977
TNF	0.05	0.71445	0.587115	0.452641	0.520232
PDGF	0.05	0.05	0.05	0.05	0.05
IFN	0.05	0.05	0.05	0.05	0.05

C) IRF/ ISGF3 activation profile

	0 min	**30 min**	**120 min**	**240 min**	**480 min**
LPS	0.05	0.05	0.444394	0.641238	0.109184
TNF	0.05	0.05	0.05	0.0853709	0.070391
PDGF	0.05	0.0647129	0.05	0.05	0.05
IFN	0.05	0.99917	1.	0.66436	0.323157

Table S2, related to Figure 5. TF input profiles for BMDM GRNs,
Table of nuclear TF activities, determined by quantifying immunoblots (p-cJun) and EMSAs (NFκB and ISF3), and normalizing basal and maximum activity values to 0.05 and 1, respectively.

A) AP1 activation profile

	0 min	30 min	60 min	120 min	240 min	1440 min
LPS	0.05	1	0.1	0.05	0.05	0.05
TNF	0.05	0.5	0.1	0.05	0.05	0.05
IFNB	0.05	0.05	0.05	0.05	0.05	0.05
PAM	0.05	0.75	0.2	0.05	0.05	0.05
PIC	0.05	0.5	0.2	0.05	0.05	0.05
FLA	0.05	0.5	0.1	0.05	0.05	0.05
FSL	0.05	0.5	0.2	0.05	0.05	0.05
R848	0.05	0.5	0.2	0.05	0.05	0.05
CPG	0.05	0.5	0.2	0.05	0.05	0.05
Trans PIC	0.05	0.3	0.1	0.05	0.05	0.05
MCMV	0.05	0.05	0.05	0.05	0.05	0.05
VSV	0.05	0.1	0.05	0.05	0.05	0.05
STREP	0.05	0.5	0.1	0.05	0.05	0.05
LM	0.05	0.5	0.1	0.05	0.05	0.05

B) NFκB activation profile

	0 min	30 min	60 min	120 min	240 min	1440 min
LPS	0.05	0.5	1	0.5	0.5	0.2
TNF	0.05	1	0.25	0.5	0.25	0.05
IFNB	0.05	0.05	0.05	0.05	0.05	0.05
PAM	0.05	1	0.25	0.5	0.5	0.2
PIC	0.05	0.05	0.5	0.2	0.2	0.2
FLA	0.05	0.1	1	0.2	0.2	0.05
FSL	0.05	0.3	0.5	0.75	0.3	0.2
R848	0.05	0.3	0.5	1	0.5	0.2
CPG	0.05	0.1	1	0.5	0.5	0.1
Trans PIC	0.05	0.05	0.5	0.5	0.2	0.2
MCMV	0.05	0.05	0.05	0.1	0.1	0.05
VSV	0.05	0.05	0.5	0.2	0.2	0.2
STREP	0.05	0.3	0.5	1	0.5	0.1
LM	0.05	0.3	0.5	1	0.5	0.1

C) IRF/ ISGF3 activation profile

	0 min	60 min	120 min	180 min	1440 min
LPS	0.05	1	1	0.5	0.2
TNF	0.05	0.05	0.05	0.05	0.05
IFNB	0.05	1	1	0.8	0.2
PAM	0.05	0.05	0.05	0.1	0.05
PIC	0.05	0.5	1	0.5	0.2
FLA	0.05	0.05	0.05	0.05	0.05
FSL	0.05	0.1	0.1	0.05	0.05
R848	0.05	0.1	0.1	0.05	0.05
CPG	0.05	0.2	0.1	0.1	0.05
Trans PIC	0.05	1	0.8	0.8	0.2
MCMV	0.05	0.1	0.1	0.5	0.05
VSV	0.05	0.2	0.5	1	0.2
STREP	0.05	0.1	0.1	0.1	0.05
LM	0.05	0.05	0.1	0.1	0.05

Table S3, related to Figure 2. Expression data for LPS-inducible gene clusters in MEFs, stimulated with cytokines.
The Table provides the normalized expression data for 714 genes (log2 induction folds relative unstimulated) for indicated stimulation conditions and timepoints , as well as their cluster assignment.

Table S4, related to Figure 3. Expression data for LPS-inducible gene clusters in MEFs deficient for NFκB and IRF transcription factors.
The Table provides the expression values for 714 genes (log2 induction folds relative unstimulated) measured in MEFs of indicated genotypes, in indicated stimulation conditions and timepoints.

Table S5, related to Figure 5. Expression data for LPS-inducible gene clusters in BMDMs, stimulated with cytokines.
The Table provides the expression values (log2 induction folds relative to unstimulated) for 782 genes for indicated stimulation conditions and timepoints, as well as their cluster assignment.

Table S6, related to Figure 6. Sequence-Based Reagents.
Sequences of qPCR primers used to detect mature or nascent mRNA of indicated genes.

GCCACCACGCTCTTCTGTCT	this paper (mature)	Tnfa.f
GAGGCCATTTGGGAACTTCT	this paper (mature)	Tnfa.r
CTGGCCACAGGGGCGCCTATC	this paper (mature)	Cxcl1.f
AATCCCAGCCATGGTCTGCAGGC	this paper (mature)	Cxcl1.r
TCCAGAGCTTGAGTGTGACG	this paper (mature)	Cxcl2.f
GCCCTTGAGAGTGGCTATGA	this paper (mature)	Cxcl2.r
TGCCATCTACGAGAGCCTCCAGT	this paper (mature)	TTP.f
CCGAGGGATTCGGTTCCTCCGT	this paper (mature)	TTP.r
GTGGCCTTGGGCCTCAAAGGAAA	this paper (mature)	Il1b.f
GGATCCACACTCTCCAGCTGCAGG	this paper (mature)	Il1b.r
AGCAGCCATGCCCCTATGGGAA	this paper (mature)	Zc3h12a.f
CTTGGCCGCTCAGGGTGGAA	this paper (mature)	Zc3h12a.r
TCTCTGCAAGAGACTTCCATCCAGT	this paper (mature)	Il6.f
AGTAGGGAAGGCCGTGGTTGTCA	this paper (mature)	Il6.r
TGTTCCTACCCCCAATGTGT	this paper (mature)	Gapdh.f
CATACTTGGCAGGTTTCTCC	this paper (mature)	Gapdh.r
CCCAGACCCTCACACTCAGTA	this paper (nascent)	nt Tnfa.f
AACTGCCCTTCCTCCATCTT	this paper (nascent)	nt Tnfa.r
CCGCTGACCAAGAGTCTTCT	this paper (nascent)	nt Cxcl1.f
CATGGTCTGCAGGCACTGAC	this paper (nascent)	nt Cxcl1.r
TCCAGAGCTTGAGTGTGACG	this paper (nascent)	nt Cxcl2.f
AGAGGCTATCCAGGGAGACA	this paper (nascent)	nt Cxcl2.r
TCTCTTCACCAAGGCCATTC	this paper (nascent)	nt TTP.f
CGGGATGCTGTCTAACAGG	this paper (nascent)	nt TTP.r
TGAAGCAGCTATGGCAACTG	this paper (nascent)	nt Il1b.f
CCACTCTCCAGTACCCACTGA	this paper (nascent)	nt Il1b.r
CGACCAGATGTGCCTATCAC	this paper (nascent)	nt Zc3h12a.f
TTAAGGACCCTTTGGGGAAG	this paper (nascent)	nt Zc3h12a.r
GCCCAACTGTGCTATCTGCT	this paper (nascent)	nt Il1b.f
TCAGTCCCAAGAAGGCAACT	this paper (nascent)	nt Il1b.r
CTGTATTCCCCTCCATCGTG	this paper (nascent)	nt actin.f
GCTTGCCACTCCCAAAGTAA	this paper (nascent)	nt actin.r