Cell, Volume *138*

Supplemental Data

Control of Inducible Gene

Expression by Signal-Dependent

Transcriptional Elongation

Diana C. Hargreaves, Tiffany Horng, and Ruslan Medzhitov

Figure S1: S5P Pol II, S2P Pol II ChIP.
(A, B) BMMΦs were analyzed by ChIP (S5P Pol II, S2P Pol II). Genes are shaded and ranked as housekeeping (HKG), CpG-rich primary (PRG-I), CpG-poor primary (PRG-II), or secondary genes (SRG). Data are representative of 3 or more independent experiments. Error bars represent mean+/-SEM.

Figure S2: cdk9 recruitment in Brd4 knockdown cells.
(A) BMMΦs were transfected with siRNA oligos targeted to Brd4 (d1,d2) or a scrambled control oligo (sc), stimulated for 1 or 2 hours with LPS, and analyzed by ChIP (cdk9). Data are representative of 3 or more independent experiments.

Figure S3: The basal state of LPS-inducible genes in MEFs.
(A-D) MEFs were analyzed by ChIP (H3K9Ac, H3K4me3, S5P Pol II, Sp1). (E, F) MEFs were analyzed by ChIP (H4K5Ac, H4K12Ac). Genes are shaded and ranked as housekeeping (HKG) or genes induced by LPS in macrophages. Data are representative of 3 or more independent experiments. Error bars represent mean+/-SEM.

Figure S1

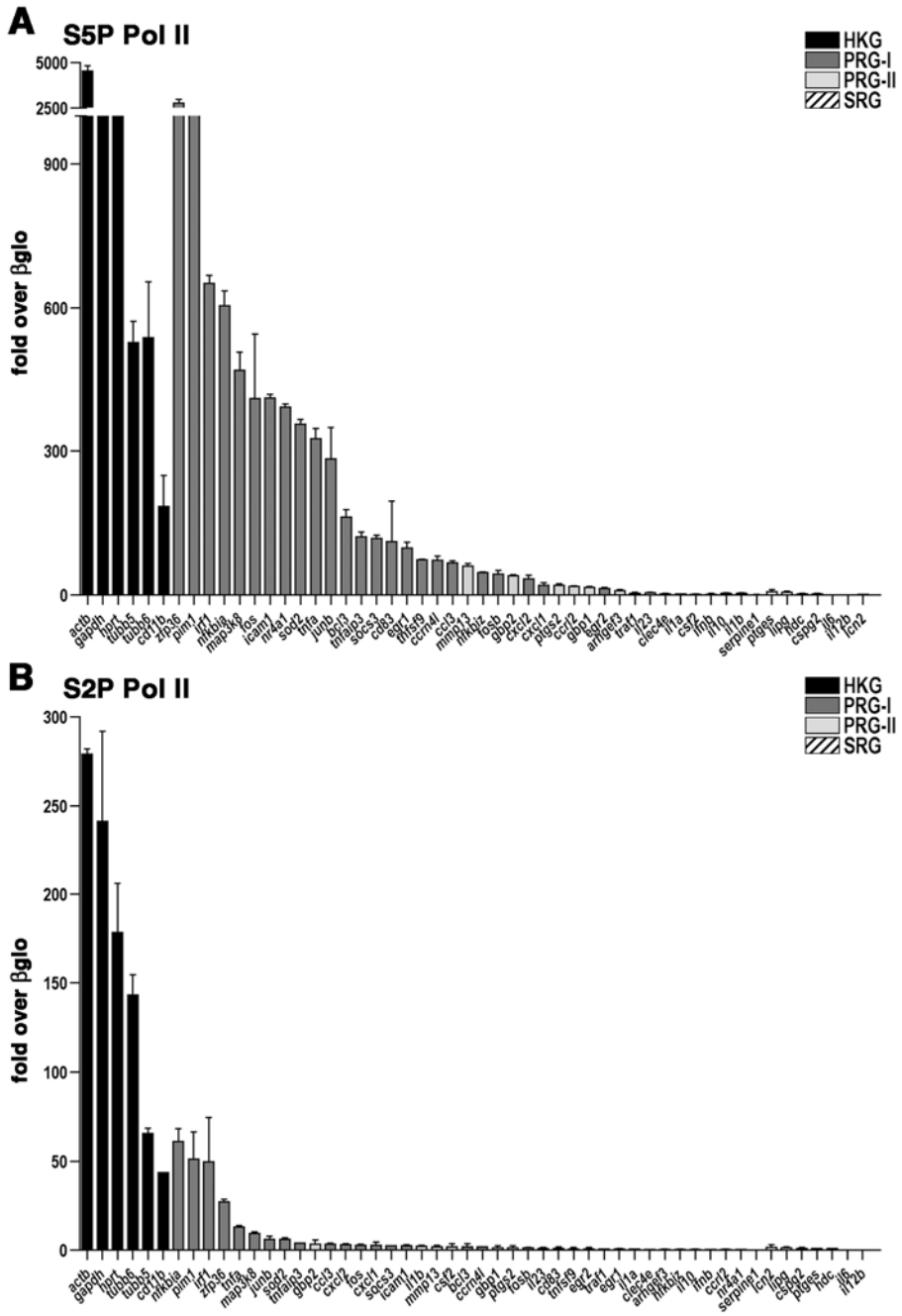
A
S5P Pol II
HKG
PRG-I
PRG-II
SRG
fold over βglio
5000
2500
900
600
300
0
actb gapdh hprt tubb5 tubb6 cd11b nfkbia pim1 irf1 zfp36 tnfa mapk8 fos icam1 nr4a1 socs2 tnfa junb dcn3 tnfaip3 socs3 cd83 egr1 tnfrsf9 ccnd4l ccl3 mmp13 nfkbiz fosb gbp2 cxcr2 cxcl1 ptgs2 ccr2 gbp1 egr2 arhgef3 traf1 il23 clec4e il1a csf2 ifnb il10 il1b serpine1 ptges hpg hdc cspg2 il6 il12b icn2
B
S2P Pol II
HKG
PRG-I
PRG-II
SRG
fold over βglio
300
250
200
150
100
50
0
actb gapdh hprt tubb6 tubb5 cd11b nfkbia pim1 irf1 zfp36 tnfa mapk8 junb socs2 tnfaip3 gbp2 ccl3 cxcr2 fos cxcl1 socs3 icam1 il1b mmp13 csf2 dcn3 ccnd4l gbp1 ptgs2 fosb il23 cd83 tnfrsf9 egr2 traf1 egr1 il1a clec4e arhgef3 nfkbiz il10 ifnb ccr2 nr4a1 serpine1 icn2 hpg cspg2 ptges hdc il6 il12b

Figure S2

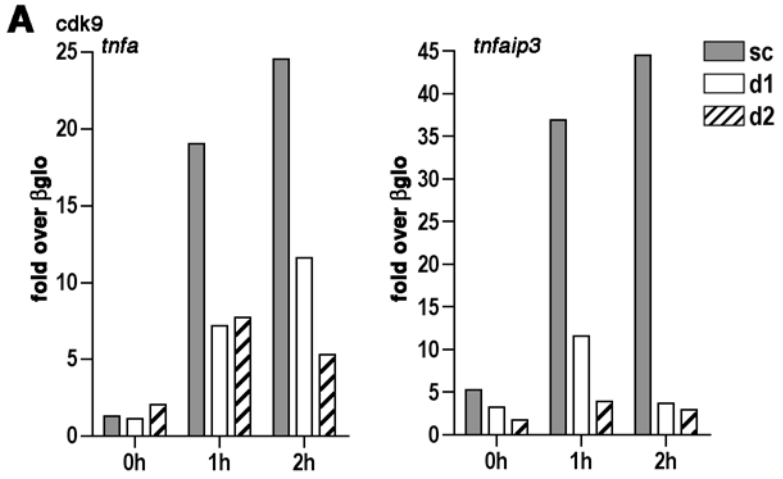
A
cdk9
tnfa
fold over βglo
25
20
15
10
5
0
0h
1h
2h
tnfaip3
fold over βglo
45
40
35
30
25
20
15
10
5
0
0h
1h
2h
sc
d1
d2

Figure S3

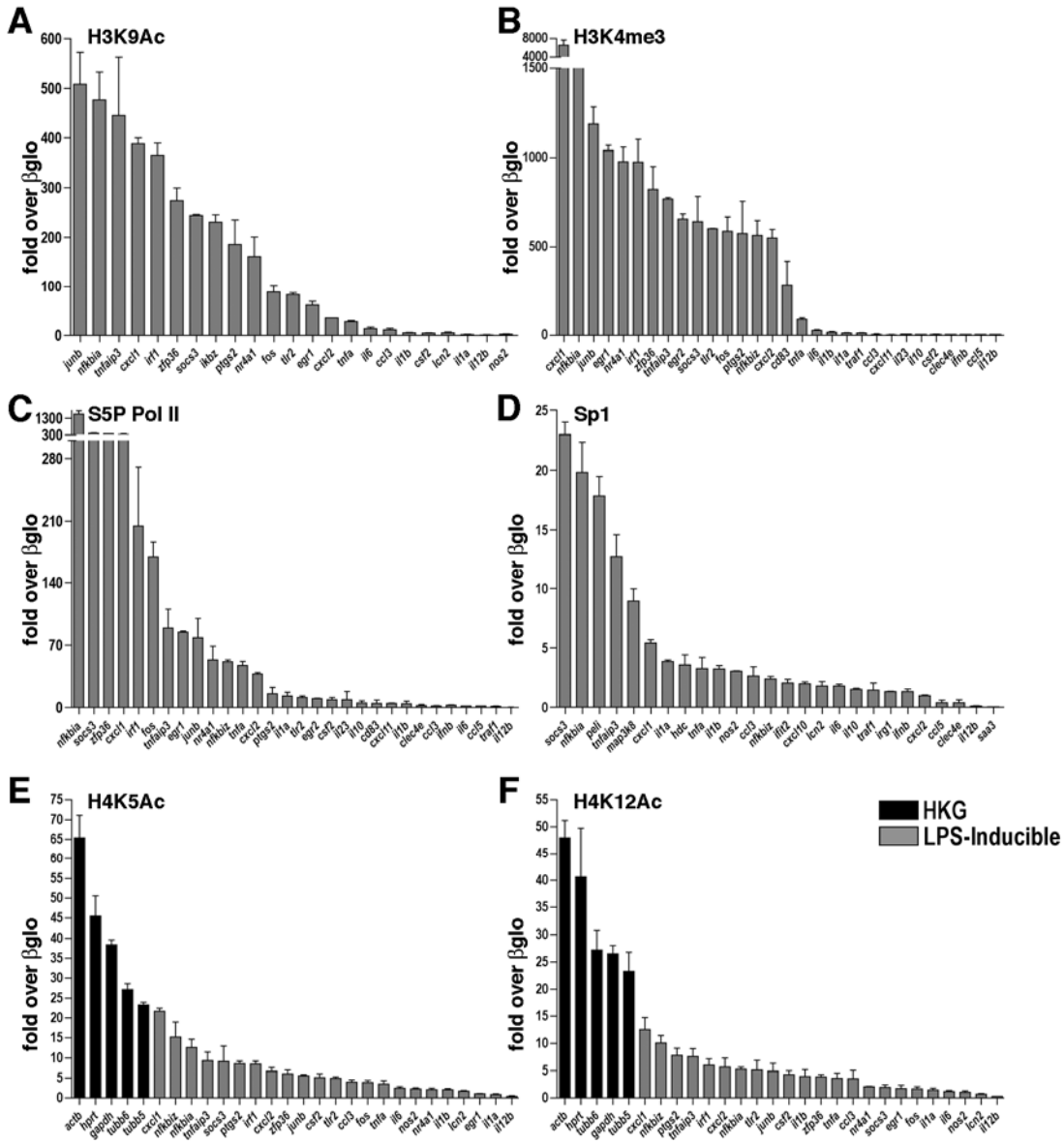
A
H3K9Ac
fold over βglo
B
H3K4me3
fold over βglo
C
S5P Pol II
fold over βglo
D
Sp1
fold over βglo
E
H4K5Ac
fold over βglo
F
H4K12Ac
fold over βglo
HKG
LPS-Inducible

Table S1: Genes analyzed for Figure 7F.

PRG-I	PRG-II/SRG
cd83	ccrl2
nr4a1	traf1
ccrn4l	clec4e
irf1	il1a
nfkbiz	csf2
sod2	il23a
pim1	ccl3
socs3	gbp1
tnfaip3	il1b
egr1	map3k8
junb	serpine1
marcksl1	arhgef3
bcl3	vcam1
icam1	saa3
zfp36	ccl2
tnfsf9	il10
nfkbia	irg1
fosb	ikbke
egr2	ccl12
fos	mmp13
ptgs2	il18
tlr2	csprs
cxcl1	il12b
cxcl2	plcb4
	nfxl1
	mthfr
	syk
	phlpp
	nr1h3
	dst
	gda
	klf1b
	mf24
	ralgds
	lf2ak2
	crk7
	lphn2
	rnf14
	brwd3

Supplemental Experimental Procedures

Table S2: List of antibodies used in this study.

Antibody	Catalog number	Company
H3Ac	06-599	Millipore
H3K4me3	07-473	Millipore
H4K5Ac	07-327	Millipore
H4K8Ac	07-328	Millipore
H4K12Ac	07-595	Millipore
H3K27me3	07-499	Millipore
Sp1	17-601	Millipore
P50	06-886	Millipore
CoREST	07-455	Millipore
HDAC1	06-720	Millipore
RNA Pol II	Sc-899X	Santa Cruz
Cdk9	Sc-8338X	Santa Cruz
Cyclin T1	Sc-10750X	Santa Cruz
P300/CBP	Sc-585X	Santa Cruz
PCAF	Sc-8999X	Santa Cruz
GCN5	Sc-20698X	Santa Cruz
P65	Sc-372X	Santa Cruz
HDAC3	Sc-11417X	Santa Cruz
S5P Pol II	Ab5131	Abcam
S2P Pol II	Ab5095	Abcam
Brd4	Ab46199	Abcam
NCoR	PA1-844A	Affinity Bioreagents
Actin	A3853	Sigma
GST	G7781	Sigma

Reverse transcription and Quantitative PCR (qPCR). Total RNA was isolated with RNA-bee reagent (Tel-Test). Total RNA was reverse transcribed with an oligo (dT) primer or random primers (precursor, unspliced transcripts) using Superscript reverse transcriptase III (Invitrogen). cDNA was analyzed by qPCR amplification using SYBR Green QPCR Master Mix (Qiagen) on the Eppendorf realplex System (Eppendorf). The PCR amplification conditions were: 95°C (15 min), 45 cycles of 94°C (15s), 58°C (15s), and 72°C (40s). Primer pairs were designed within a single 3' exon (precursor), across the last intron/exon border (unspliced), or spanning an intron (spliced) and unique products were tested by melt-curve analysis. To analyze precursor, unspliced, and spliced transcripts in untreated BMMΦs (Figure 1B, 3), Ct values were converted to copy number using standard curves. For all other Figures, data was analyzed by Relative Quantitation with untreated values set as calibrator and expression normalized to *hprt* or *rpl13a*. Data is represented as the fold induction over untreated and displayed as mean+/- SEM. Primer sequences are available upon request.