Cell Reports

Supplemental Information

Cooperative Transcriptional Activation of Antimicrobial Genes by STAT and NF-κB Pathways by Concerted Recruitment of the Mediator Complex

Sebastian Wienerroither, Priyank Shukla, Matthias Farlik, Andrea Majoros, Bernadette Stych, Claus Vogl, HyeonJoo Cheon, George R. Stark, Birgit Strobl, Mathias Müller, and Thomas Decker

Figure S1

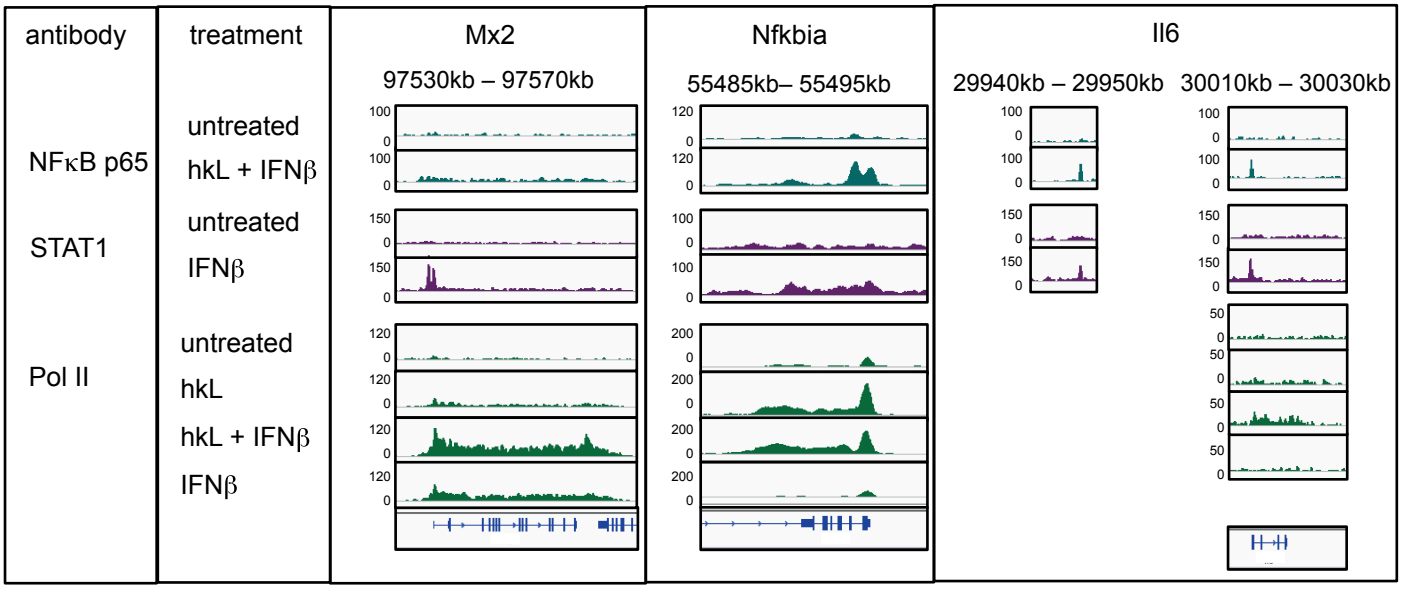

antibody
treatment
Mx2
97530kb – 97570kb
Nfkbia
55485kb– 55495kb
Il6
29940kb – 29950kb
30010kb – 30030kb
NFκB p65
STAT1
Pol II
untreated
hkL + IFNβ
untreated
IFNβ
untreated
hkL
hkL + IFNβ
IFNβ

Figure S2

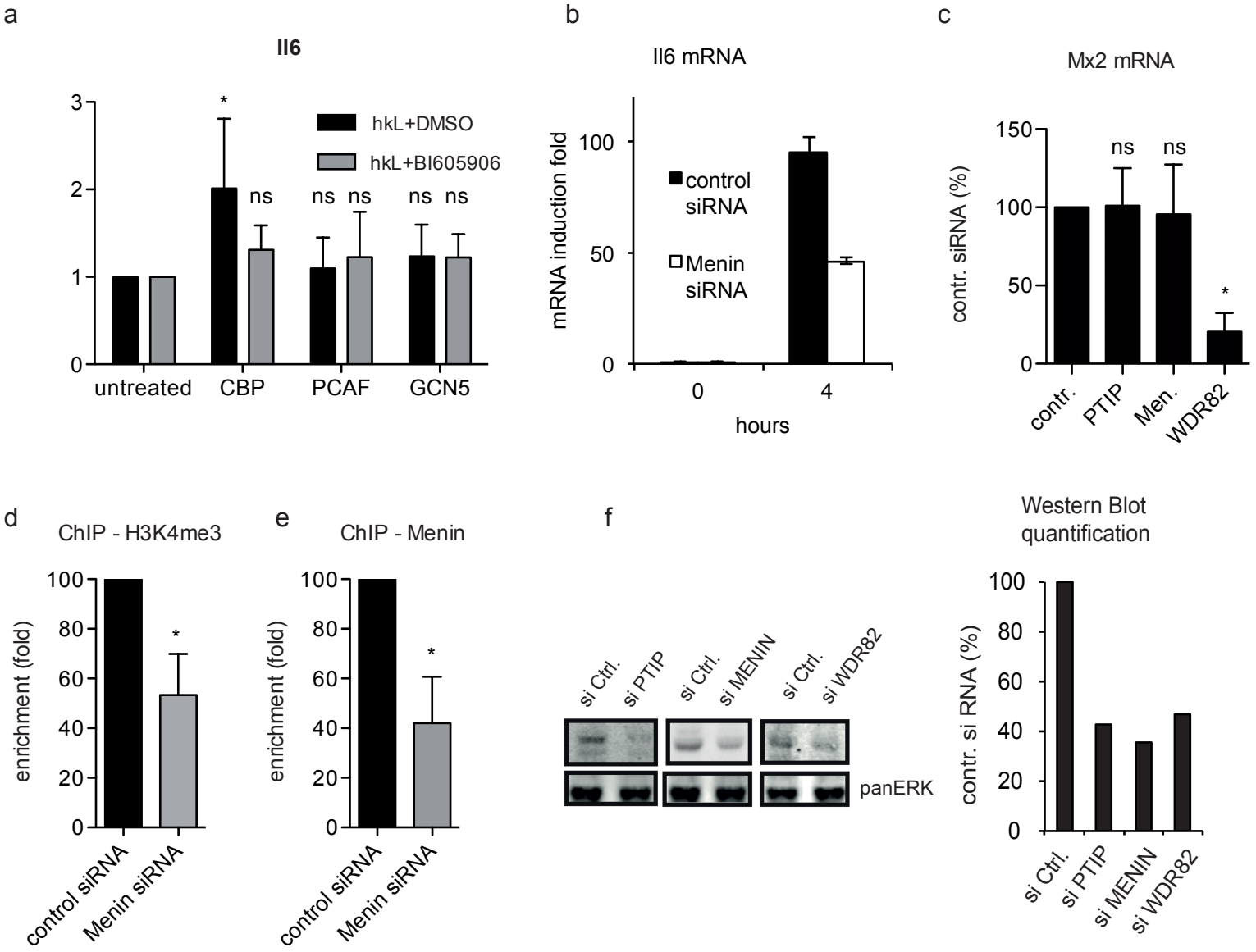

a
Il6
hkL+DMSO
hkL+BI605906
untreated
CBP
PCAF
GCN5
ns
b
Il6 mRNA
mRNA induction fold
control siRNA
Menin siRNA
hours
c
Mx2 mRNA
contr. siRNA (%)
contr.
PTIP
Men.
WDR82
d
ChIP - H3K4me3
enrichment (fold)
control siRNA
Menin siRNA
e
ChIP - Menin
f
Western Blot quantification
si Ctrl.
si PTIP
si MENIN
si WDR82
panERK
contr. si RNA (%)

Figure S3

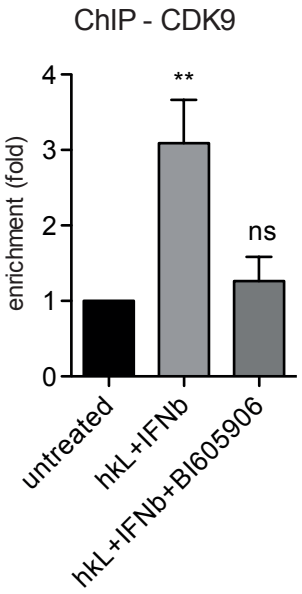

ChIP - CDK9
enrichment (fold)
4
3
2
1
0
**
ns
untreated
hkL+IFNb
hkL+IFNb+BI605906

Figure S4

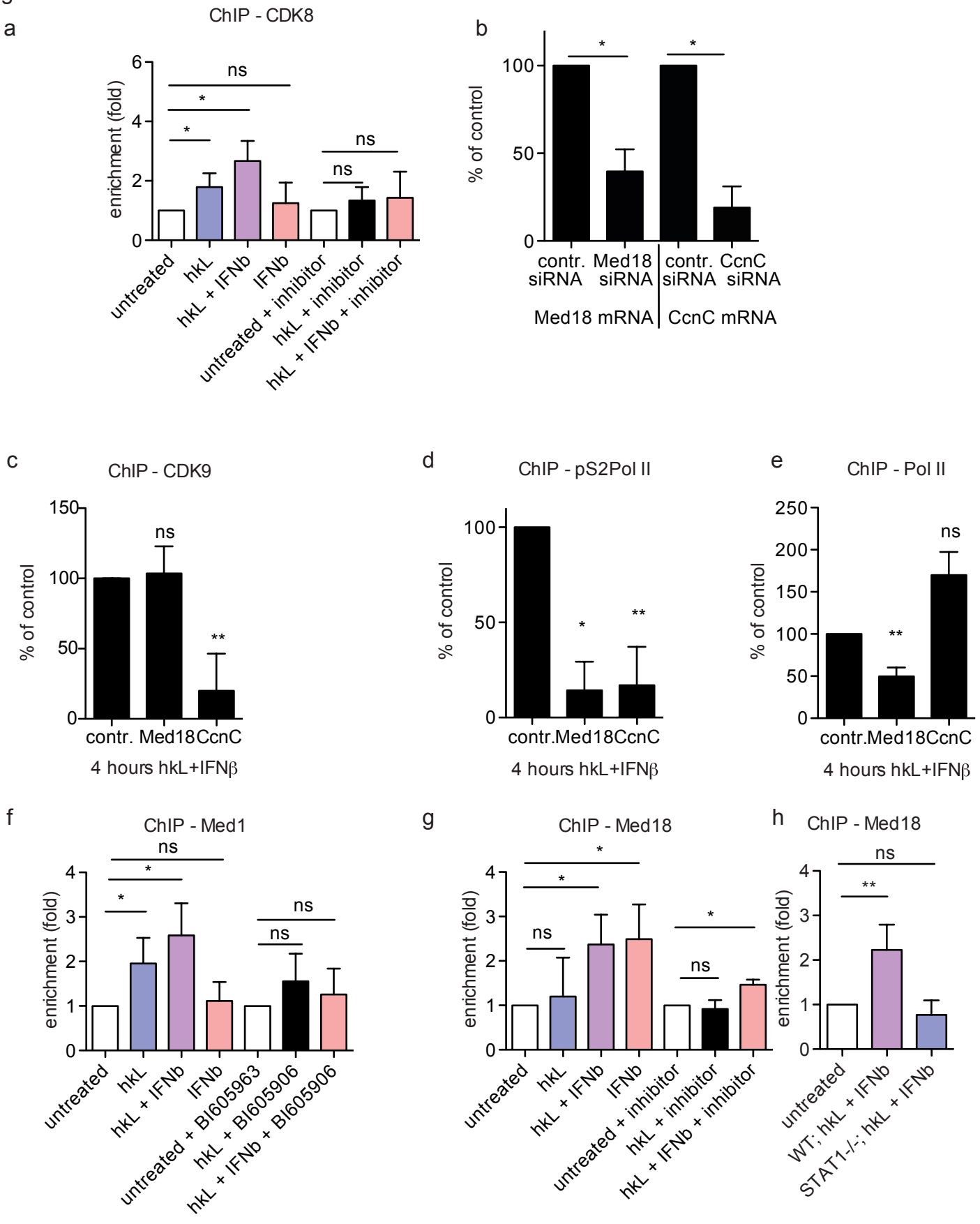

a
ChIP - CDK8
enrichment (fold)
ns
*
*
ns
ns
untreated
hkL
hkL + IFNb
IFNb
untreated + inhibitor
hkL + inhibitor
hkL + IFNb + inhibitor
b
% of control
*
*
contr. siRNA
Med18 siRNA
contr. siRNA
CcnC siRNA
Med18 mRNA
CcnC mRNA
c
ChIP - CDK9
% of control
ns
**
contr.
Med18
CcnC
4 hours hkL+IFNβ
d
ChIP - pS2Pol II
% of control
*
**
contr.
Med18
CcnC
4 hours hkL+IFNβ
e
ChIP - Pol II
% of control
**
ns
contr.
Med18
CcnC
4 hours hkL+IFNβ
f
ChIP - Med1
enrichment (fold)
ns
*
*
ns
ns
untreated
hkL
hkL + IFNb
IFNb
untreated + BI605963
hkL + BI605906
hkL + IFNb + BI605906
g
ChIP - Med18
enrichment (fold)
*
*
ns
*
ns
untreated
hkL
hkL + IFNb
IFNb
untreated + inhibitor
hkL + inhibitor
hkL + IFNb + inhibitor
h
ChIP - Med18
enrichment (fold)
ns
**
untreated
WT; hkL + IFNb
STAT1-/-; hkL + IFNb

Figure S5

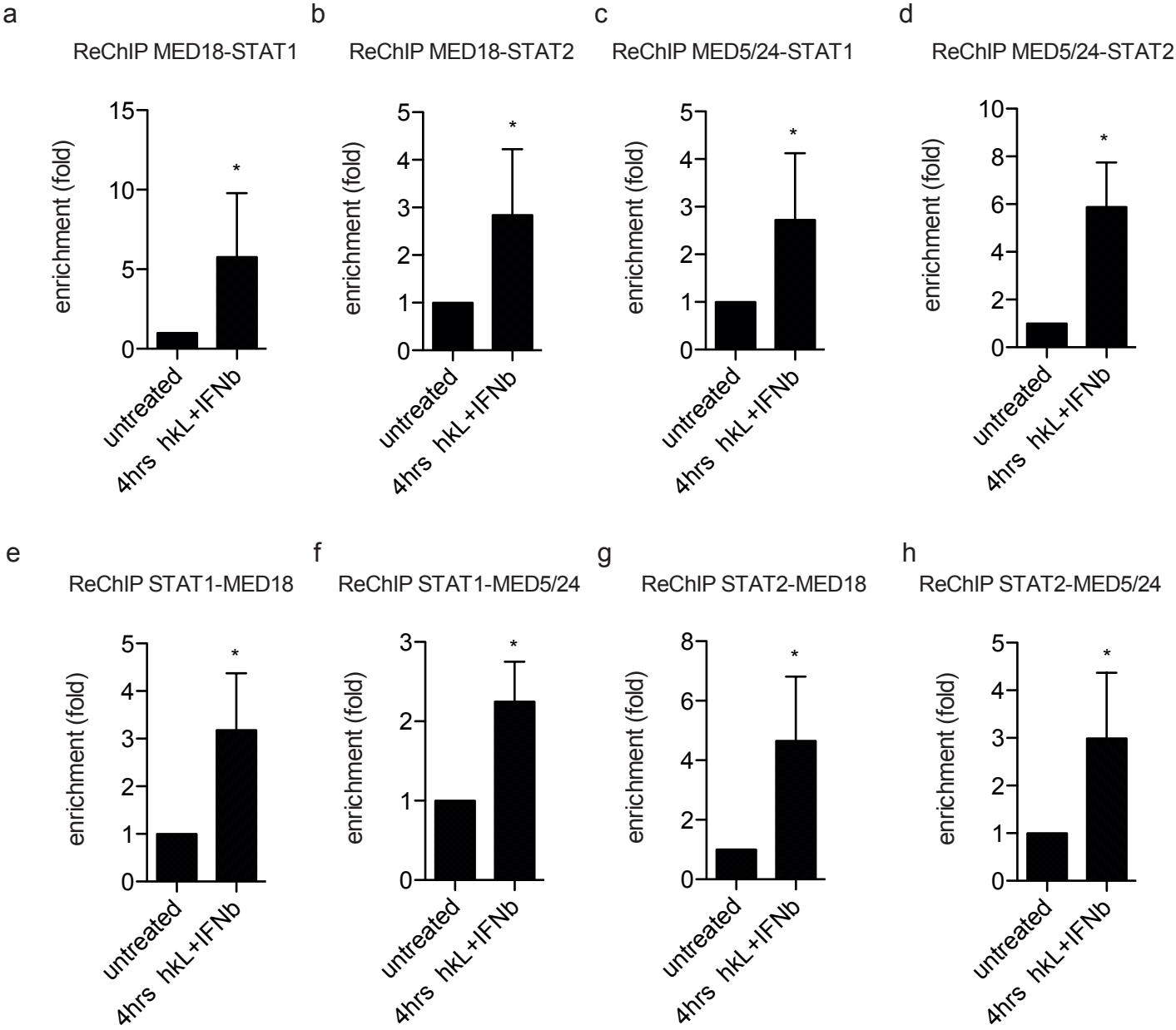
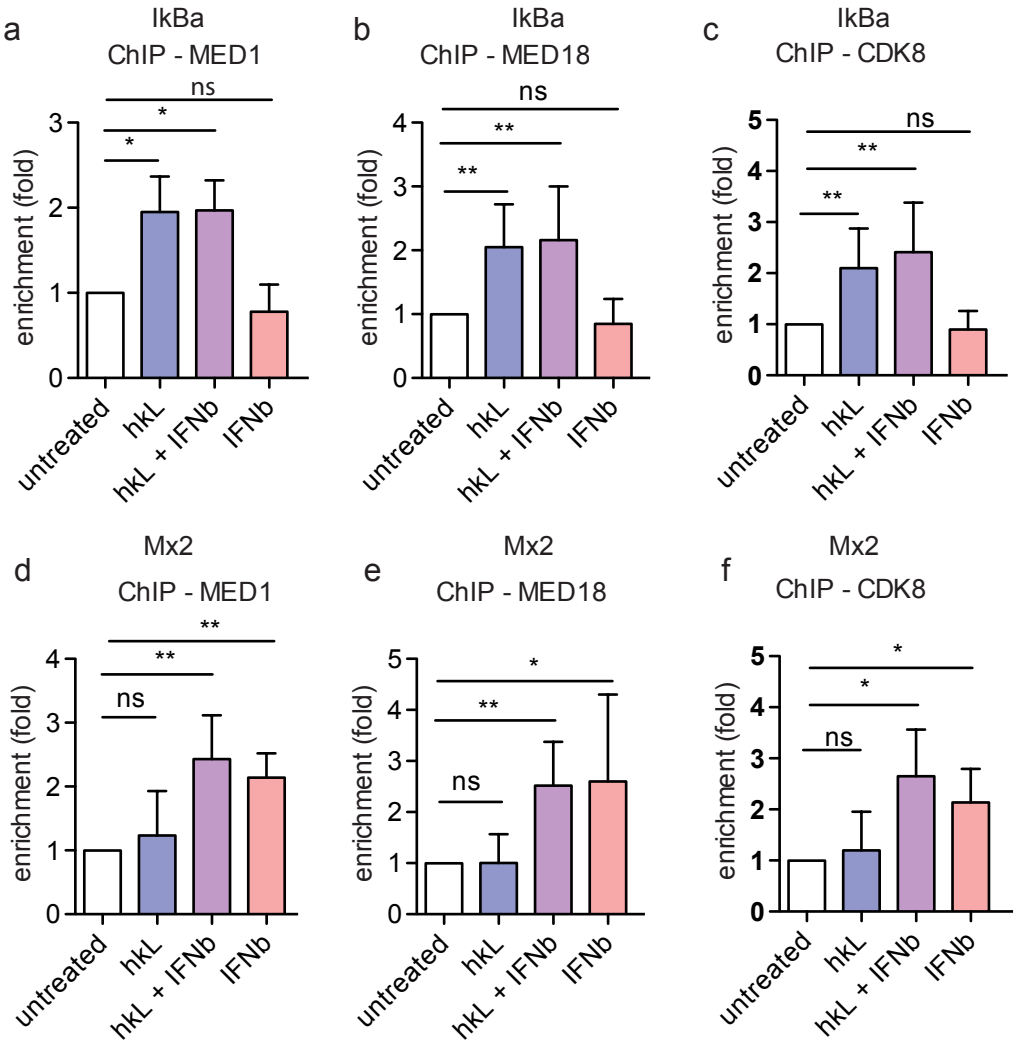

a
ReChIP MED18-STAT1
b
ReChIP MED18-STAT2
c
ReChIP MED5/24-STAT1
d
ReChIP MED5/24-STAT2
e
ReChIP STAT1-MED18
f
ReChIP STAT1-MED5/24
g
ReChIP STAT2-MED18
h
ReChIP STAT2-MED5/24
enrichment (fold)
untreated
4hrs hkL+IFNb
*

Figure S6


a
IkBa
ChIP - MED1
b
IkBa
ChIP - MED18
c
IkBa
ChIP - CDK8
d
Mx2
ChIP - MED1
e
Mx2
ChIP - MED18
f
Mx2
ChIP - CDK8
enrichment (fold)
ns
*
**
untreated
hkL
hkL + IFNb
IFNb

Supplementary figure legends

Figure S1, related to figure 2: ChIP-Seq analysis NFκB and ISGF3 target genes. BMDMs were treated with heat-killed Listeria (hkL), IFNβ, or with a combination of both for 4 hours. For STAT1 ChIP treatment was with IFNβ for two hours. Cells were processed for ChIP with indicated antibodies (Pol II, NFκBp65, STAT1), followed by next generation sequencing of the precipitate (ChIP-Seq). Genome browser views of the results obtained for the Mx2 (left panel), Nfκbia (middle panel) and Il6 (right panel) genes are shown.

Figure S2, related to figure 3: Histone modifiers at the Il6 promoter. (a) BMDMs were treated with hkL with or without 10μM IKKβ inhibitor BI605906 for 4 hours followed by ChIP with antibodies to CBP, PCAF and GCN5. DNA encompassing the TSS was amplified. (b-e) BMDM were transfected with Menin siRNA and treated with hkL+IFNβ for 4 hours. Il6 (b) and Mx2 (c) mRNA expression was analyzed by Q-PCR (b, c), H3K4me3 modification (d) and Menin binding (e) were determined by ChIP, amplifying the region encompassing the TSS. (f) western blot to assess the knock-down efficiency (right panel shows the quantification by densitometry).

Figure S3, related to figure 4: CDK9 recruitment to the Il6 promoter. BMDMs were treated with hkL+IFNβ for 4 hours with (dark grey) or without (light grey) 10 μM IKKβ inhibitor BI605906 followed by ChIP using antibody to CDK9. DNA encompassing the TSS was amplified

Figure S4, related to figure 4, 5 and 6: Knockdown of MED18 and CcnC. (a) WT BMDMs were treated with hkL or IFNβ or a combination of both for 4 hours, with and without 10 μM IKKβ inhibitor BI605906 as indicated, followed by ChIP analysis with antibody to CDK8 and Q-PCR for promoter region of Il6. (b) Knockdown was performed in BMDMs using siRNA as described in material and methods. The cells were lysed and mRNA analysis (b) was performed to analyze the knockdown efficiency. A knockdown of MED18 or CcnC in BMDMs was performed and cells were treated with hkL and IFNβ for 4 hours followed by ChIP with antibodies to CDK9 (c), pS2Pol II (d) and Pol II (e). WT and STAT1 -/- BMDMs were treated with hkL or IFNβ or a combination of both for 4 hours, with and without 10μM IKKβ inhibitor BI605906 as indicated, followed by ChIP analysis with antibody to MED1 (f) and MED18 (g, h) and Q-PCR for promoter region of Il6. For all ChIP experiments DNA encompassing the TSS was amplified. Values represent means and standard errors of at least three independent biological replicates. *, P<0,05; **, P<0,01; ***, P<0,001

Figure S5, related to figure 6. ChIP-re-ChIP analysis of Stat1, Stat2 and core mediator binding to the Nos2 promoter. WT BMDMs were treated with hkL and IFNβ for 4 hours, followed by ChIP with the antibodies to MED18 (a, b), MED5/24 (c, d), STAT1 (e, f), and STAT2 (g, h). The samples were re-precipitated with STAT1 (a, c), STAT2 (b, d), MED18 (e, g) and MED5/24 (f, h). The re-precipitates were analyzed by Q-PCR, amplifying the 209bp of the Nos2 promoter including the IFN response region (imperfect ISRE sequences at -952/-940 and -924/-911 with respect to the TSS).

Figure S6, related to figures 4, 5 and 6: Mediator subunit recruitment to the IκBα and Mx2 genes. BMDMs were treated with hkL+IFNβ for 4 hours followed by ChIP analysis with antibodies to MED1, MED18 and CDK8 (a,b). IκBα (a) and Mx2 proximal promoter regions (b) including the TSS were analyzed for protein binding. Values represent means and standard errors of at least four independent biological replicates. *, P<0,05; **, P<0,01; ***, P<0,001

Gene	NFκB binding site	STAT1 binding site
1190002H23Rik	-	-
1190003J15Rik	-	-
Adora2b	+	-
Arg2	-	-
Ccl7	+	+
Ccrl2	+	+
Cd40	-	+
Cd83	+	+
Ch25h	+	+
Cxcl9	-	+
Dgka	-	-
Dusp2	-	+
Edn1	+	+
Egr1	-	-
Gpr109a	-	-
Hdc	-	+
Il10	+	+
Il15ra	+	+
Il19	-	-
Il1a	-	-
Il1rn	+	+
Il27	+	+
Il6	+	+
Indo	-	-
Inhba	+	-
Irf8	-	+
Mxd1	-	+
Nos2	+	+
Nrg1	-	+
Prokr1	-	-
Ptx3	-	-
S100a8	-	-
Serpinb2	-	+
Slamf1	+	+
Slc28a2	+	+
Slc6a4	-	-
Tagap	+	+
Thbs1	-	-
Tmtc2	+	+
Tpbg	-	-
Upp1	-	-
Vcam1	-	-

Table S1, related to figures 1 and 2: mRNAs displaying synergistic induction by type I IFN and heat-killed Listeria (hkL) as determined by microarray, related to figures 1 and 2. The presence of binding sites for NFκB/p65 and STAT1 within 50kB of the transcription start (TSS) of the corresponding genes was determined by ChIP-Seq. For further explanation see text.

a) Contingency tables were computed for the NFκB_STAT1 pair, depending on the presence or absence of at least one ChIP-Seq peak 50kb upstream of the transcription start or within the gene, and on the presence or absence of at least 2-fold synergistic enrichment of Pol II with the double treatment versus single treatment samples.

NFκB	STAT1	Pol II syn.	Pol II not syn.	Total
absent	absent	103	26950	27053
present	absent	35	568	603
absent	present	123	5964	6087
present	present	127	1089	1216
Total		388	34571	34959

b) Many hypotheses tests are possible with this table; the two used in the main text are presented. For genes with Pol II synergism, the presence or absence of NFκB and STAT1 peaks produce the following 2x2 contingency table:

	STAT1 absent	STAT1 present	Total
NFκB absent	103	123	226
NFκB present	35	127	162
Total	138	250	388

c) Analysis of genes without and with synergy for the presence or absence of concomitant NFκB and STAT1 peaks results in the following 2x2 contingency table:

	Pol II syn.	Pol II not syn.	Total
NFκB or STAT1 absent	261	33482	33743
both NFκB and STAT1 present	127	1089	1216
Total	388	34571	34959

Table S2, related to figure 2. Association of STAT1 and NFκB binding with synergistic gene induction by heat-killed *L. monocytogenes* and type I IFN.

Table S3, related to figure 2: GO-analysis and KEGG pathway of 127 genes with STAT1 and NFκB binding sites showing synergistic NFκB and ISGF3 activity according to RNA polymerase II signals in ChIP-Seq. Untreated cells are compared with cells subjected to single IFNβ or hkL treatment, or with cells treated with combined hkL+IFNβ.

Antibody	Catalog number	Company
STAT1	sc-346	Santa Cruz
CDK9	sc-484	Santa Cruz
H3	ab1791	Abcam
H4ac	06-866	Upstate
H4K5ac	Kind gift from Christian Seiser	
H4K8ac	Kind gift from Christian Seiser	
H4K12ac	Kind gift from Christian Seiser	
H4K16ac	Kind gift from Christian Seiser	
Med1	sc-5334	Santa Cruz
Med4	Ab129170	Abcam
Med7	sc-12457	Santa Cruz
CDK8	sc-1521	Santa Cruz
Med18	A300-777A	Bethyl
Med24	A301-472A	Bethyl
Med26	sc-48776	Santa Cruz
CcnC	sc-1061	Santa Cruz
Pol II	sc-899	Santa Cruz
pS2Pol II	A300-654A	Bethyl
CBP	sc-369	Santa Cruz
GCN5	sc-20698x	Santa Cruz
PCAF	sc-8999x	Santa Cruz

Table S4, related to figures 2-6. Antibodies used for ChIP

Q-PCR

Nos2 for	GCTTGCCCCAACAGGAGAAG
Nos2 rev	GCTGCCCGGAAGGTTTGTAC
Il6 for	CTGCAAGAGACTTCCATCCAG
Il6 rev	AGTGGTATAGACAGGTCTGTTGG
Il1rn for	GTGCTACTGGGGCTCATTTGT
Il1rn rev	GGAGTAAGAGGACACTTGCGAAT
Ccrl2 for	CCCCGGACGATGAATATGATG
Ccrl2 rev	CACCAAGATAAACACCGCCAG
HPRT for	GTTGGATACAGGCCAGACTTTGTTG
HPRT rev	GAGGGTAGGCTGGCCTATTGGCT

ChIP

Nos2 prx for	GGTCCCAGTTTTGAAGTGACTACG
Nos2 prx rev	GTTGTGACCCTGGCAGCAG
Nos2 dis for	CCAACTATTGAGGCCACACAC
Nos2 dis rev	GCTTCCAATAAAGCATTCACA
Mx2 for	ACCCAGCCAAGGCCCCCTTA
Mx2 rev	GCAGCTGCCAGGGCTCAGAC
IkBa for	AAGAAGGGTTCTTGCAGAGGGCT
IkBa rev	TCGTCCTCCACTGAGAAGCCTAAA
Il6 for	ATCCAGTTGCCTTCTTGGGACTGA
Il6 rev	ATCAGTTTCACAGCCTACCCACCT
Nos2 30kb distal for	CTGGAGTCTGTTCTTCTGACTG
Nos2 30kb distal rev	CAAGAGGCCACAAGAGAAT

Table S5, related to figures 1-6. Primer sequences used for gene expression and ChIP analysis by Q-PCR in 5' -> 3' polarity