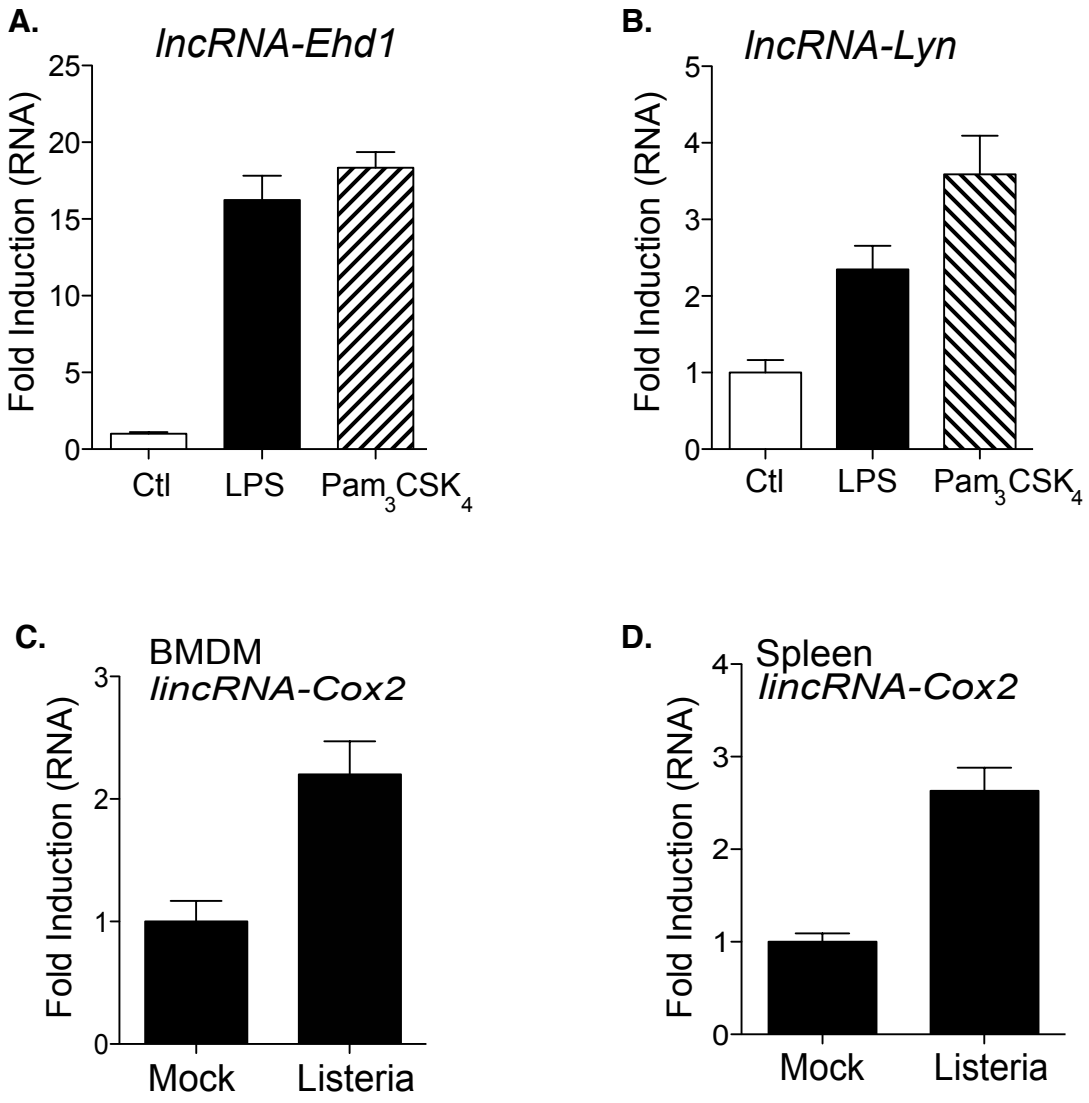
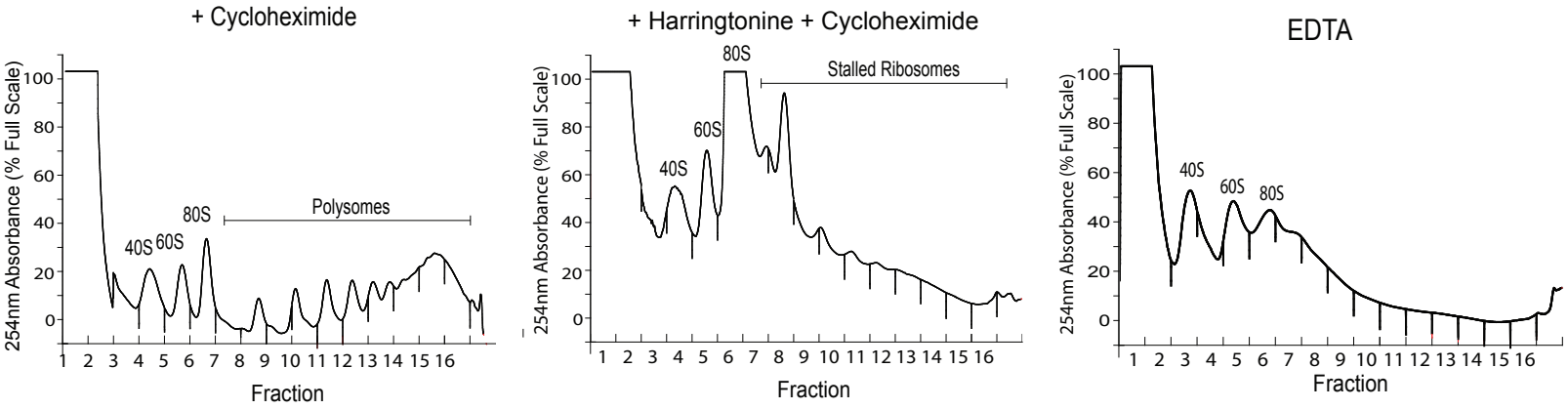
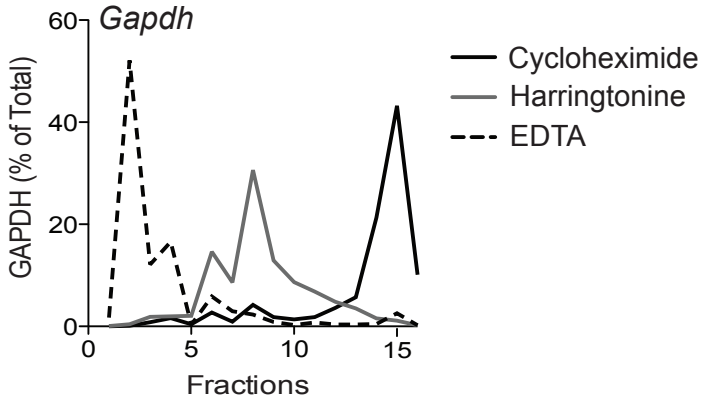
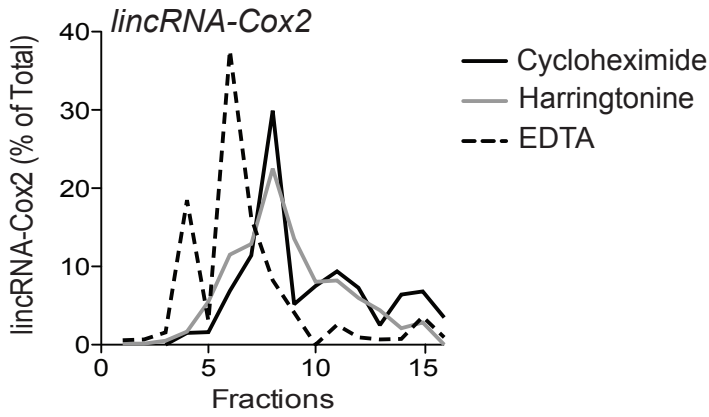

A.
lncRNA-Ehd1
Fold Induction (RNA)
25
20
15
10
5
0
Ctl
LPS
Pam3CSK4
B.
lncRNA-Lyn
Fold Induction (RNA)
5
4
3
2
1
0
Ctl
LPS
Pam3CSK4
C.
BMDM
lincRNA-Cox2
Fold Induction (RNA)
3
2
1
0
Mock
Listeria
D.
Spleen
lincRNA-Cox2
Fold Induction (RNA)
4
3
2
1
0
Mock
Listeria

Fig. S1


+ Cycloheximide
254nm Absorbance (% Full Scale)
40S
60S
80S
Polysomes
Fraction
+ Harringtonine + Cycloheximide
Stalled Ribosomes
EDTA


Gapdh
GAPDH (% of Total)
Fractions
Cycloheximide
Harringtonine
EDTA


lincRNA-Cox2
lincRNA-Cox2 (% of Total)
Fractions
Cycloheximide
Harringtonine
EDTA

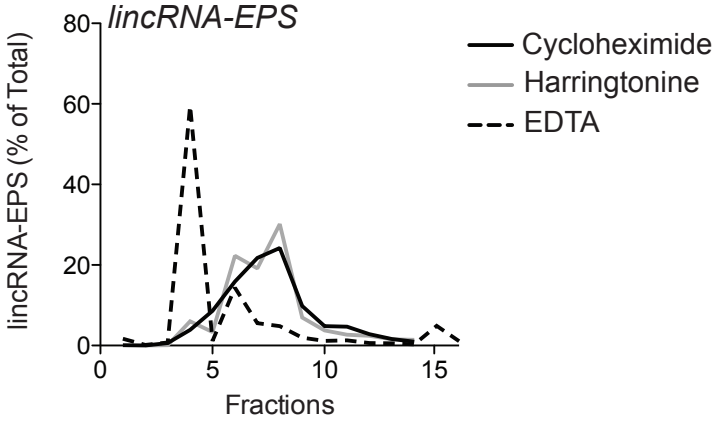
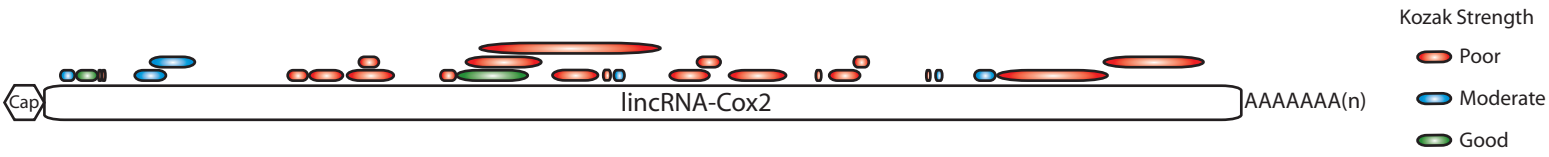

lincRNA-EPS
lincRNA-EPS (% of Total)
Fractions
Cycloheximide
Harringtonine
EDTA

fig. S2

(A)



(B)

Position	Nucleotide Sequence	Peptide Sequence	Predicted Molecula	Kozak Strength
29-46	atgcgcgggacattctga	MRGTF*		Moderate
53-103	atgcccagagacaaaaagga	MPRDKKEAWRCEK/		Good
103-111	atgagttga	MS*		Very Poor
111-116	atgtga	M*		Very Poor
142-186	atgctgtactcttggaactttct	MLYSWNFLDKRKHA		Moderate
179-229	atgcataagactcctcacctct	MHKTPHLSASAHKT		Moderate
370-396	atgtggtatctaacaggcaca	MWYLTGTG*		Very Poor
401-448	atgagccctatggaagagctg	MSPMEELWAGQPK		Moderate-Poor
456-524	atggcaagtaggagagcattt	MASRRAFLRESVLK		Moderate-Poor
509-514	atgtaa	M*		Very Poor
600-617	atgaaacatttaggataa	MKHLG*		Poor
617-724	atggctagcagaaaatttgaa	MASRKFESQESESF		Good
681-746	atgaatctgaatgtatatttccc	MNLNVYFPSWKLFT		Poor
691-921	atgtatatttcccttcctggaaa	MYISLPGNFSLASKH		Very Poor
750-833	atgtatgtcagtgttctttgcct	MYVSVLCLSFTHFC		Very Poor
836-850	atgctttcattttaa	MLSF*		Poor
866-898	atgaggctccttcagattgttca	MRLLQIVHSN*		Moderate
942-1004	atgcagatagcttttgcaacta	MQIAFATMWCFLGT		Very Poor
1001-1015	atgatagagccctaa	MIEP*		Very Poor
1029-1115	atgtgcatattgttagttgttgg	MCILLVVGWLLLFV		Very Poor
1157-1165	atgccctaa	MP*		Poor
1184-1225	atgataactttatcctttcccct	MITLSFPPQFFLI*		Poor
1222-1239	atgatgttaaaagattga	MMLKD*		Poor
1326-1331	atgtga	M*		Very Poor
1342-1350	atgaactga	MN*		Moderate
1398-1427	atgaggcctcagccctacaca	MRPQPYTRN*		Moderate
1431-1598	atgctgagagtgggagaaata	MLRVGEIVLSREEHI		Very Poor
1574-1723	atgtgtgtatattatattatatta	MCVYYIILTLHIICIT		Very Poor

fig.S3

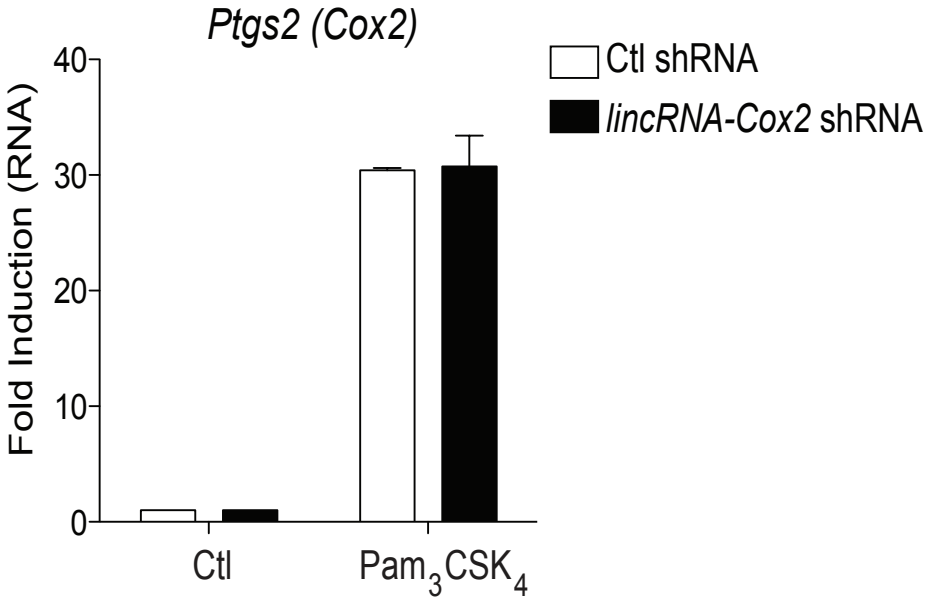
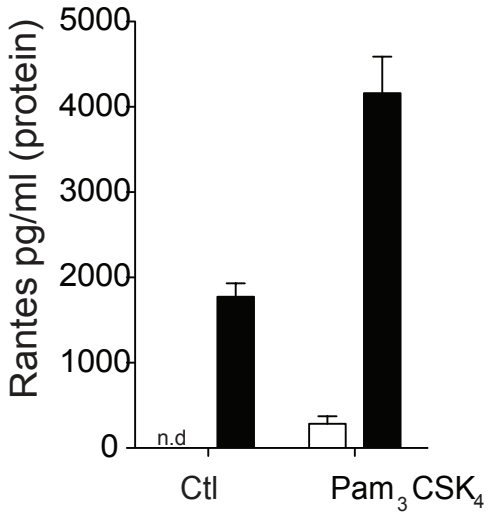

Ptgs2 (Cox2)
Ctl shRNA
lincRNA-Cox2 shRNA
Fold Induction (RNA)
40
30
20
10
0
Ctl
Pam3CSK4

fig.S4

A.


Rantes pg/ml (protein)
5000
4000
3000
2000
1000
0
n.d
Ctl
Pam3CSK4

B.

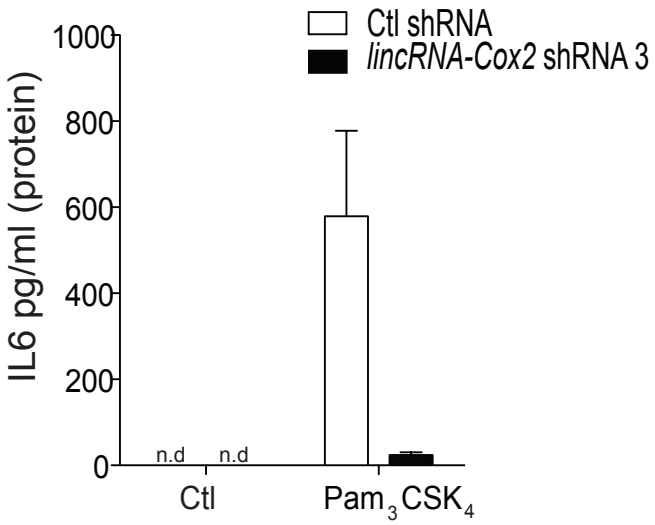

Ctl shRNA
lincRNA-Cox2 shRNA 3
IL6 pg/ml (protein)
1000
800
600
400
200
0
n.d
n.d
Ctl
Pam3CSK4

fig. S5

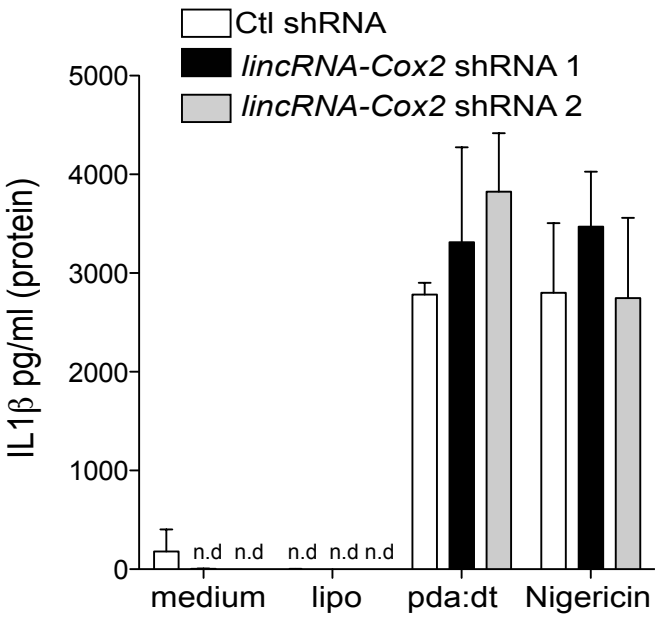

Ctl shRNA
lincRNA-Cox2 shRNA 1
lincRNA-Cox2 shRNA 2
IL1β pg/ml (protein)
5000
4000
3000
2000
1000
0
n.d
n.d
n.d
n.d
n.d
medium
lipo
pda:dt
Nigericin

Fig. S6

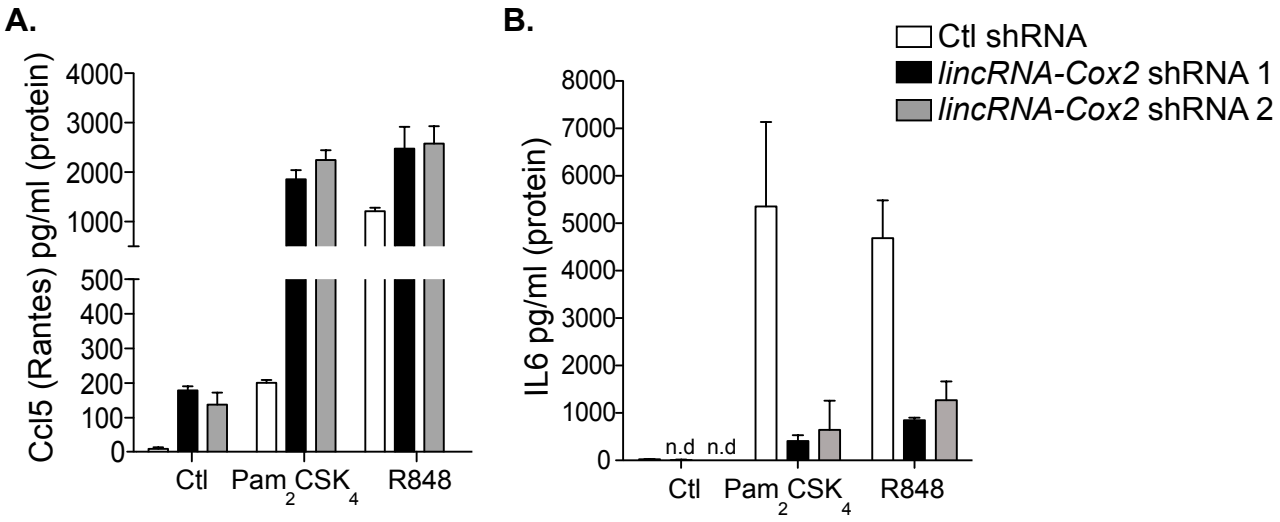
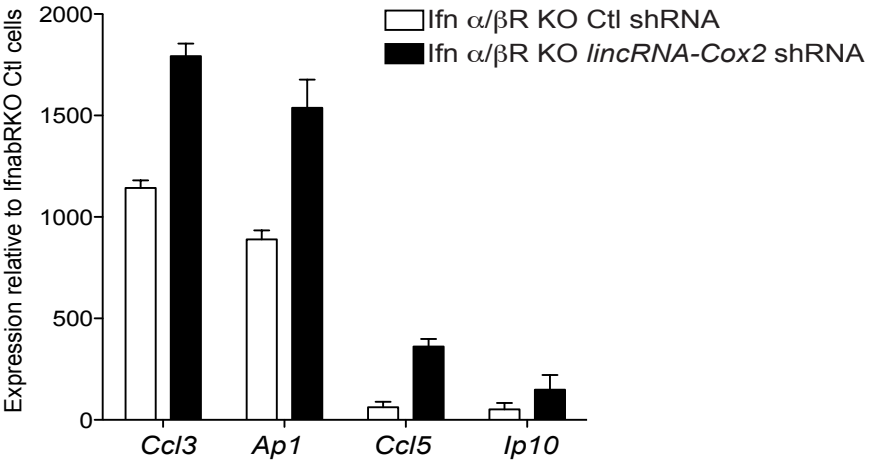

A.
Ccl5 (Rantes) pg/ml (protein)
4000
3000
2000
1000
500
400
300
200
100
0
Ctl
Pam2CSK4
R848
B.
IL6 pg/ml (protein)
8000
7000
6000
5000
4000
3000
2000
1000
0
n.d
n.d
Ctl
Pam2CSK4
R848
Ctl shRNA
lincRNA-Cox2 shRNA 1
lincRNA-Cox2 shRNA 2

Fig. S7

A.


Ifn α/βR KO Ctl shRNA
Ifn α/βR KO lincRNA-Cox2 shRNA
Expression relative to IfnabRKO Ctl cells
2000
1500
1000
500
0
Ccl3
Ap1
Ccl5
Ip10

B.

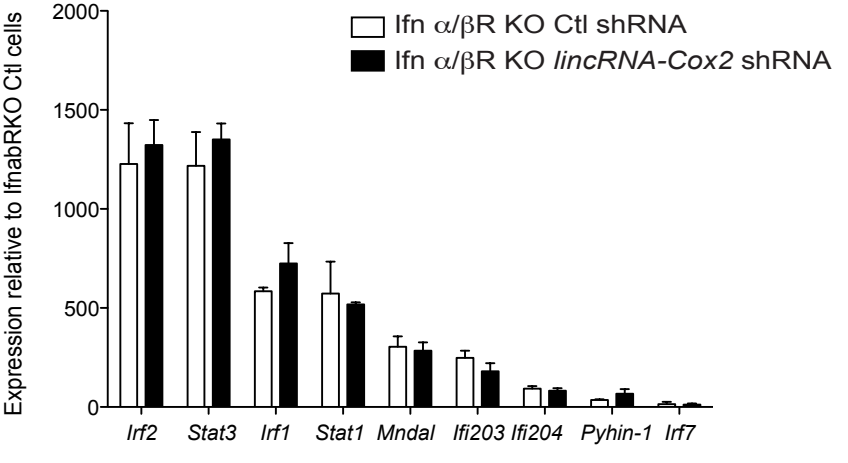

Ifn α/βR KO Ctl shRNA
Ifn α/βR KO lincRNA-Cox2 shRNA
Expression relative to IfnabRKO Ctl cells
2000
1500
1000
500
0
Irf2
Stat3
Irf1
Stat1
Mndal
Ifi203
Ifi204
Pyhin-1
Irf7

fig. S8

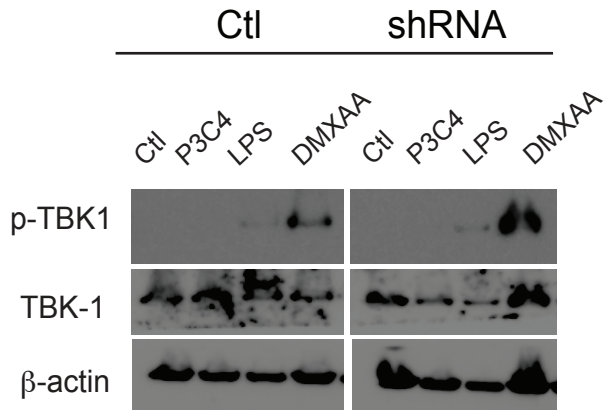

Ctl
shRNA
Ctl
P3C4
LPS
DMXAA
Ctl
P3C4
LPS
DMXAA
p-TBK1
TBK-1
β-actin

fig. S9

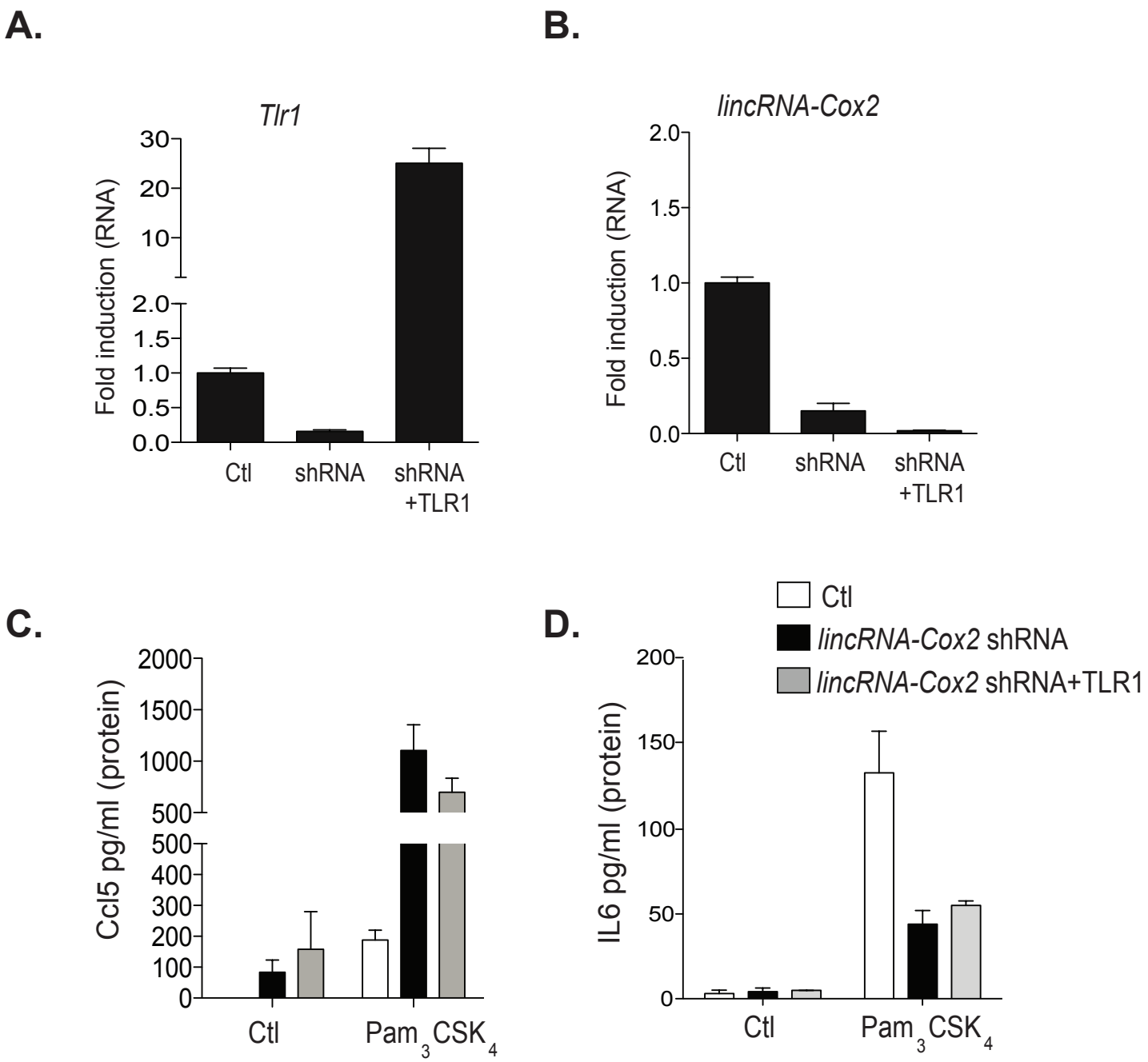

A.
Tlr1
Fold induction (RNA)
30
20
10
2.0
1.5
1.0
0.5
0.0
Ctl
shRNA
shRNA +TLR1
B.
lincRNA-Cox2
Fold induction (RNA)
2.0
1.5
1.0
0.5
0.0
Ctl
shRNA
shRNA +TLR1
C.
Ccl5 pg/ml (protein)
2000
1500
1000
500
500
400
300
200
100
0
Ctl
Pam3CSK4
D.
Ctl
lincRNA-Cox2 shRNA
lincRNA-Cox2 shRNA+TLR1
IL6 pg/ml (protein)
200
150
100
50
0
Ctl
Pam3CSK4

fig.S10

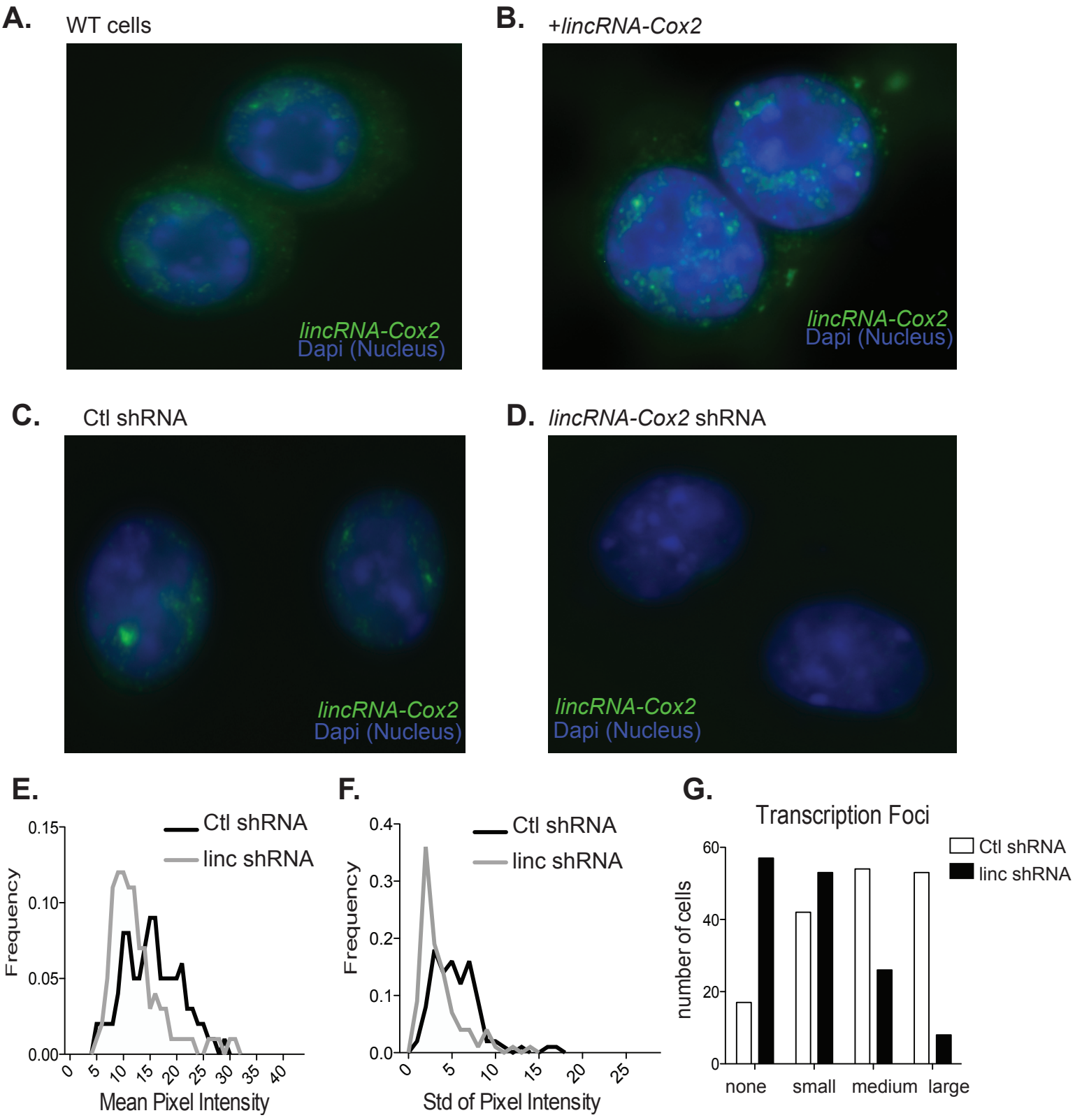

A.
WT cells
lincRNA-Cox2
Dapi (Nucleus)
B.
+lincRNA-Cox2
lincRNA-Cox2
Dapi (Nucleus)
C.
Ctl shRNA
lincRNA-Cox2
Dapi (Nucleus)
D.
lincRNA-Cox2 shRNA
lincRNA-Cox2
Dapi (Nucleus)
E.
Ctl shRNA
linc shRNA
Frequency
0.15
0.10
0.05
0.00
0 5 10 15 20 25 30 35 40
Mean Pixel Intensity
F.
Ctl shRNA
linc shRNA
Frequency
0.4
0.3
0.2
0.1
0.0
0 5 10 15 20 25
Std of Pixel Intensity
G.
Transcription Foci
Ctl shRNA
linc shRNA
number of cells
60
40
20
0
none
small
medium
large

fig. S11

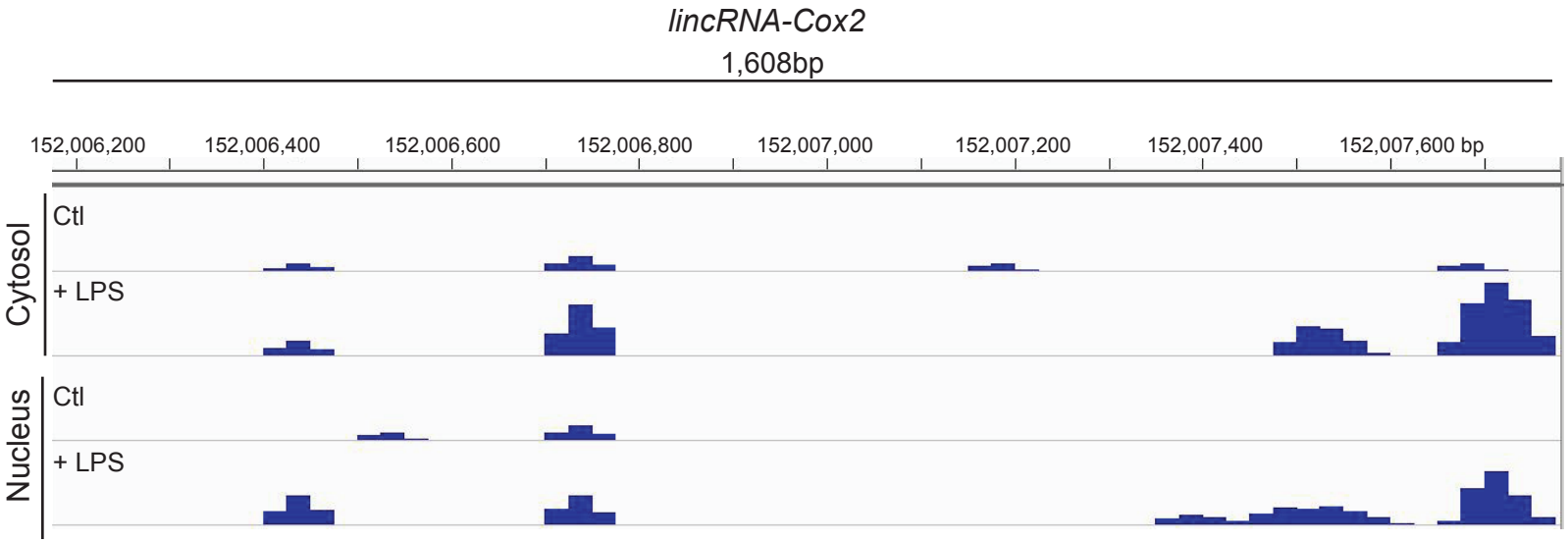

lincRNA-Cox2
1,608bp
152,006,200
152,006,400
152,006,600
152,006,800
152,007,000
152,007,200
152,007,400
152,007,600 bp
Cytosol
Ctl
+ LPS
Nucleus
Ctl
+ LPS

Fig. S12

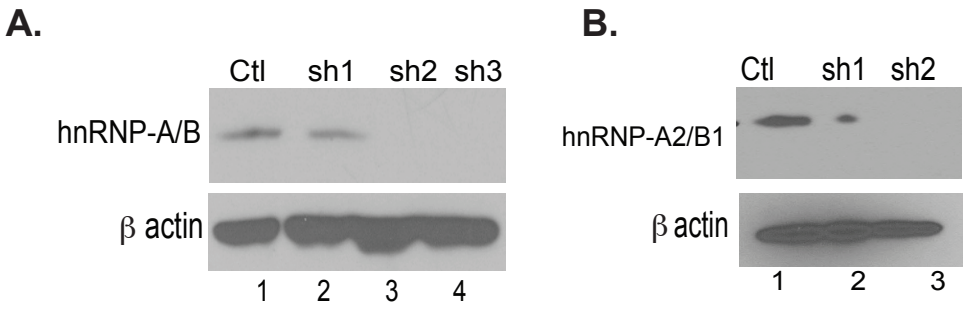

A.
Ctl
sh1
sh2
sh3
hnRNP-A/B
β actin
1
2
3
4
B.
Ctl
sh1
sh2
hnRNP-A2/B1
β actin
1
2
3

fig. S13

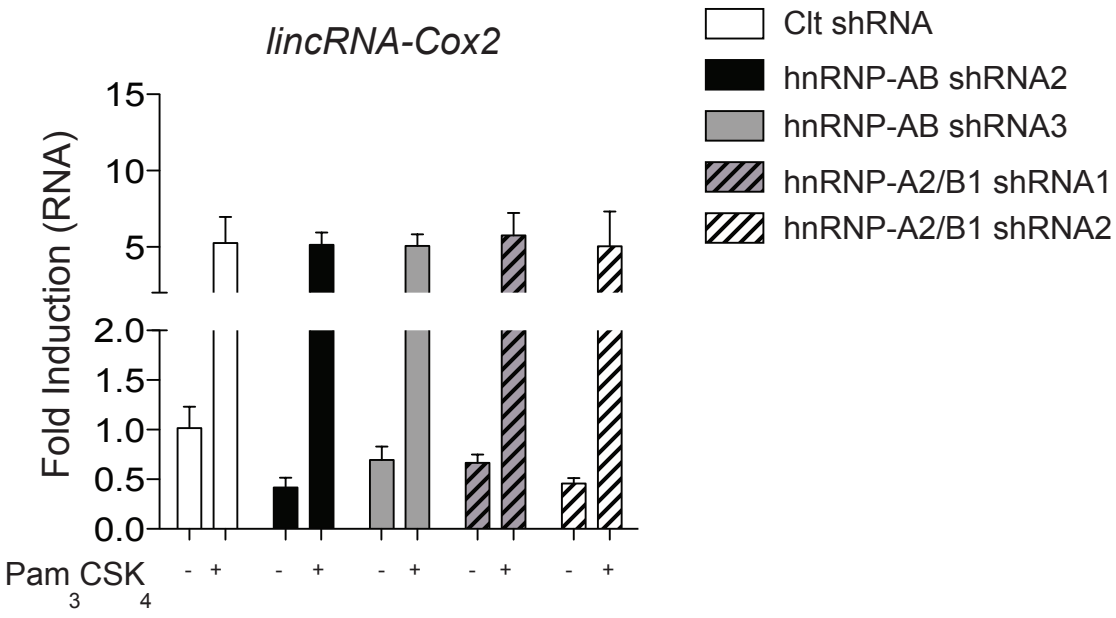
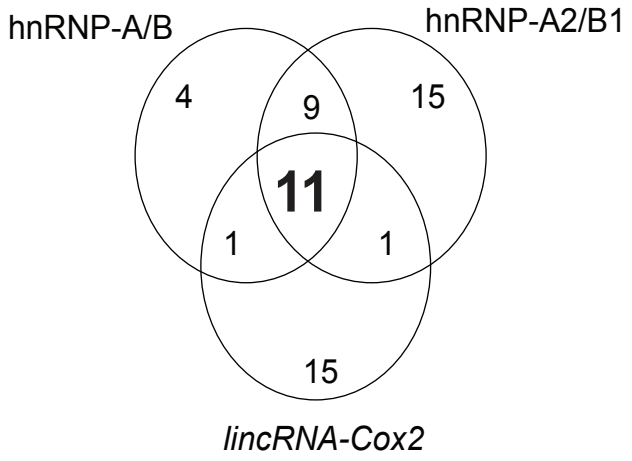
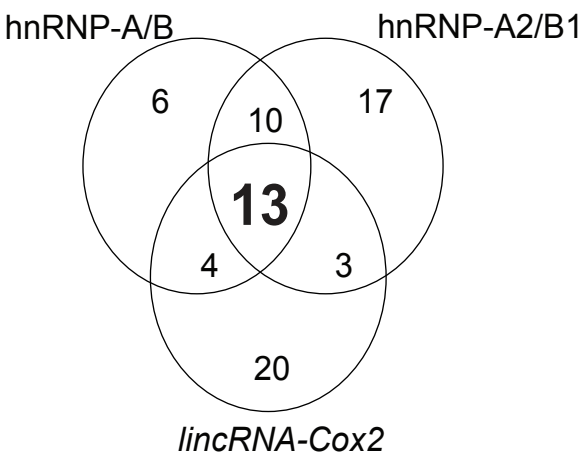

lincRNA-Cox2
Fold Induction (RNA)
15
10
5
2.0
1.5
1.0
0.5
0.0
Pam3CSK4
- + - + - + - + - +
Clt shRNA
hnRNP-AB shRNA2
hnRNP-AB shRNA3
hnRNP-A2/B1 shRNA1
hnRNP-A2/B1 shRNA2

fig.S14

A. **Basal**



B. **Pam_3CSK_4 Stimulated**

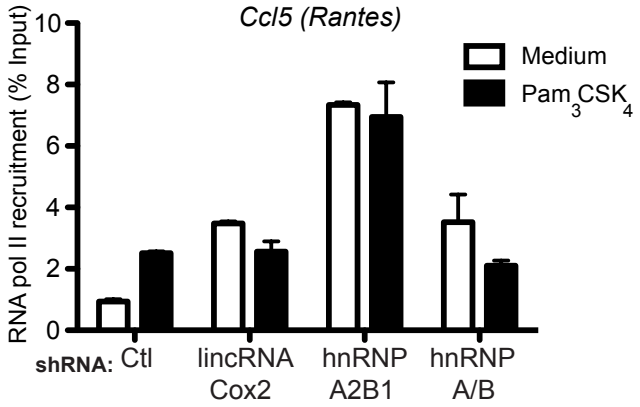


C.

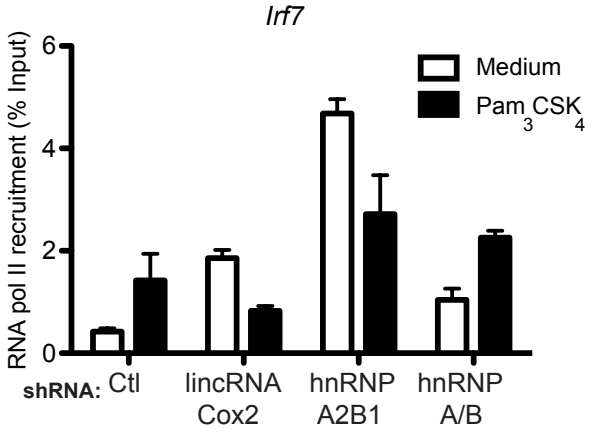
Common Genes	
Basal	**Pam_3CSK_4**
Mnda	*Mnda*
Ifi204	*Ifi204*
Ifit1	*Ifit1*
Il1RA	*Ifit2*
Stat1	*Stat1*
Ccl10	*Cxcl10*
Ccl5	*Ccl5*
Tlr8	*Nos2*
Ccl4	*Ifi205*
Casp1	*Irf5*
Prdm1	*Prdm1*
	Il1b
	Il10

fig. S15

A.


Ccl5 (Rantes)
RNA pol II recruitment (% Input)
10
8
6
4
2
0
Medium
Pam3CSK4
shRNA: Ctl
lincRNA Cox2
hnRNP A2B1
hnRNP A/B

B.


Irf7
RNA pol II recruitment (% Input)
6
4
2
0
Medium
Pam3CSK4
shRNA: Ctl
lincRNA Cox2
hnRNP A2B1
hnRNP A/B

C.

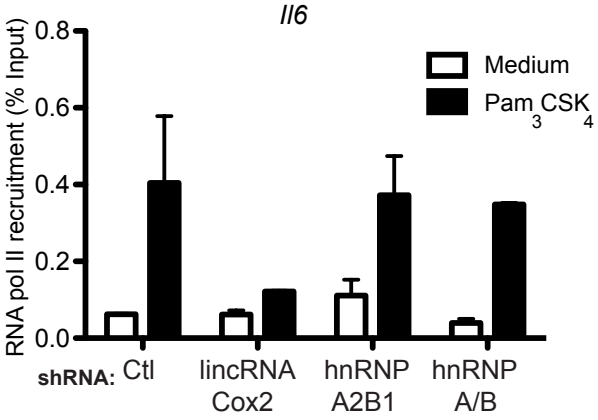
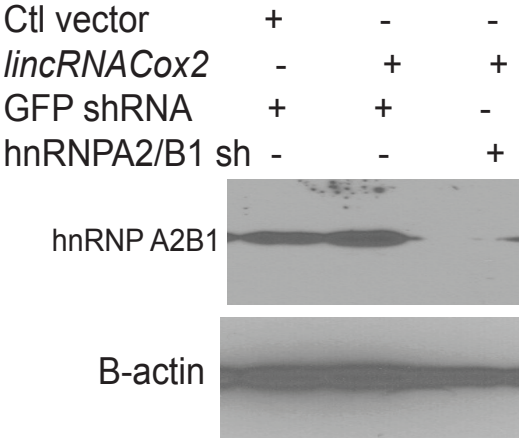

Il6
RNA pol II recruitment (% Input)
0.8
0.6
0.4
0.2
0.0
Medium
Pam3CSK4
shRNA: Ctl
lincRNA Cox2
hnRNP A2B1
hnRNP A/B

fig.S16

A.


Ctl vector + - -
lincRNACox2 - + +
GFP shRNA + + -
hnRNPA2/B1 sh - - +
hnRNP A2B1
B-actin

B.

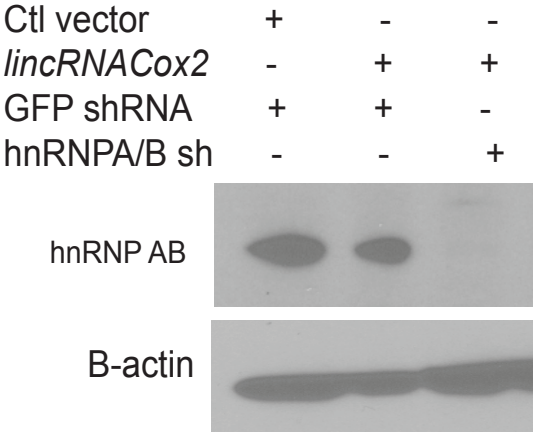

Ctl vector + - -
lincRNACox2 - + +
GFP shRNA + + -
hnRNPA/B sh - - +
hnRNP AB
B-actin

fig.S17

Supplemental Figure legends

Fig. S1. *lncRNA-Ehd1* and *lncRNA-Lyn* are inducible by LPS and Pam3CSK4, *lincRNA-Cox2* is inducible by Listeria in macrophages and spleen. qRT-PCR was carried out on primary BMDMs stimulated with LPS (TLR4) (100ng/ml) and Pam_3CSK_4 (TLR1/2) (100nM). Expression levels of *lincRNA-Ehd1*(A) and *lincRNA-Lyn* (B) were examined, normalized against *Gapdh* and expressed relative to Ctl cells (unstimulated). qRT-PCR was carried out on *in vitro* Listeria infected macrophages (BMDM) (C) and on splenocytes from mice infected with Listeria (D) *lincRNA-Cox2*, expression levels were examined.

Fig. S2. Sucrose sedimentation analysis of the cytoplasmic fraction of macrophages. A, Macrophages incubated with cycloheximide (left panel) or cycloheximide and harringtonine (middle panel) were lysed and their cytoplasmic fraction loaded on a 10-50% (weight to volume) linear sucrose gradient. The cytoplasmic fraction of untreated cells supplemented with 35 mM EDTA was also loaded on a sucrose gradient (right panel). After sedimentation, samples were fractionated while measuring absorbance at 254nm. Absorbance values are plotted from top to bottom of the sucrose gradient: fraction 1 corresponds to the top of the gradient (lightest fraction containing tRNAs and free mRNPs) while fraction 16 corresponds to the bottom of the gradient (heaviest fraction containing large polysomes). B, Analysis of RNA levels from each fraction by real-time quantitative PCR. Relative levels of *Gapdh* (top panel), *lincRNA-Cox2* (middle panel) and *lincRNA-EPS* (bottom panel) are shown for each collected fraction and condition tested.

Fig. S3. Schematic view of lincRNA-Cox2 Open Reading Frames (ORFs). A, ORFs (defined as sequences starting with an AUG triplet and ending in UAA, UAG or UGA) are depicted relative to their position within the *lincRNA-Cox2* full-length RNA and color coded with respect to the strength of the Kozak sequence surrounding the start codon.

"Poor" describes ORFs with a low likelihood of being selected by the ribosome for translation while "Good" describes those with a higher likelihood for translation. B, Position of each ORF relative to the 5' end of *lincRNA-Cox2* followed by nucleotide and amino-acid sequence, the predicted molecular weight (KDa) and the Kozak strength.

Fig. S4: Knockdown of *lincRNA-Cox2* does not affect *Ptgs2* expression. RNA analysis was carried out on BMDMs stably expressing lentiviral shRNA specific to *lincRNA-Cox2* or a Ctl shRNA. Expression of *Ptgs2 (Cox2)* was measured, normalized against housekeeping genes (*Gapdh, hprt, Gusb, Cltc, Pgk1* and *Tubb*) and expressed relative to non- stimulated control (Ctl).

Fig. S5: Knockdown of *lincRNA-Cox2* affects Pam_3CSK_4 signaling. *lincRNA-Cox2* was knocked down in macrophages using a lentiviral shRNA (shRNA 3). Cells were stimulated with Pam_3CSK_4 (TLR1/2) for 24 h, Ccl5 (Rantes) (A) and IL6 (B) cytokine levels were examined by elisa (n.d means not detected).

Fig. S6: Knockdown of *lincRNA-Cox2* does not effect IL-1β production. *lincRNA-Cox2* knockdown cells were primed with LPS (TLR4) (100ng/ml) for 3h followed by stimulation with PolydA:dT (5mg/ml) for 6h or Nigericin (10mM) for 45min, IL1β production was measured by elisa (n.d means not detected).

Fig. S7: Knockdown of *lincRNA-Cox2* results in elevated levels of Ccl5 (Rantes) and reduced levels of IL6. *lincRNA-Cox2* stable knockdown cells or their relative control were stimulated with R848 (TLR7/8) (1mg/ml, A and B) or Pam_2CSK_4 (TLR2/6) (100ng/ml, C and D) for 24 h, Ccl5 (Rantes) (A and C) and IL6 (B and D) production were examined by elisa. Data are presented as mean ± S.D of triplicate samples from one experiment representative of three experiments (n.d means not detected).

Fig. S8: Many upregulated ISGs in *lincRNA-Cox2* deficient cells are due to Type I IFNs. *lincRNA-Cox2* was silenced in interferon α/β receptor (IFN α/β R) KO cells, RNA

was extracted and subjected to non-enzymatic RNA profiling using bar-coded fluorescent probes (custom designed codeset from Nanostring Technologies). A, Expression of direct targets of *lincRNA-Cox2*: *Ccl3, Ap1, Ccl5 and Ip10* were measured B, Expression levels of indirect targets of *lincRNA-Cox2: Irf2, Stat3, Irf1, Stat1, Mndal, Ifi203, Ifi204, Pyhin-1, Irf7* were measured. All genes were normalized against housekeeping genes (*Gapdh, hprt, Gusb, Cltc, Pgk1* and *Tubb*) and expressed relative to control cells. Results represent mean ± S.D of triplicate samples.

Fig S9: Knockdown of *lincRNA-Cox2* does not affect TBK1 phosphorylation. *lincRNA-Cox2* knockdown cells or Ctl cells were stimulated with LPS (TLR4) (100ng/ml), Pam_3CSK_4 (TLR1/2) (100nM) or DMXAA (1mg/ml) for 30 min. Expression levels of phosphorylated TBK1 (top panels), total TBK1 (middle panels) and β-actin (lower panels) were determined by western blotting.

Fig. S10: Global effects of *lincRNA-Cox2* on immune genes are not solely due to the loss of TLR1 expression in *lincRNA-Cox2* deficient macrophages. *lincRNA-Cox2* knockdown cells, or cells stably reconstituted with retroviral mTLR1 were examined by qRT-PCR for expression of *Tlr1* (A) or *lincRNA-Cox2* (B). Cells were stimulated with Pam_3CSK_4 (TLR1/2) for 24 h, Ccl5 (Rantes) (C) and IL6 (D) cytokine levels were examined by elisa.

Fig. S11: *lincRNA-Cox2* is localized to both the cytosolic and nuclear compartments. A, WT BMDMs and BMDMs stably over expressing *lincRNA-Cox2* were labeled with a *lincRNA-Cox2* probe using RNA FISH, and counterstained with DAPI (DNA). B-C *lincRNA-Cox2* stable knockdown cells or Ctl cells were labeled with a *lincRNA-Cox2* probe using RNA FISH and counterstained with DAPI (DNA). D-E. Mean pixel intensity (D) and standard deviation of pixel intensity was calculated for over 100 Ctl and *lincRNA-Cox2* knockdown cells. F, Transcription foci size for Ctl and *lincRNA-Cox2* knockdown cells were determined for over 100 cells.

Fig. S12: RNA-seq reads for *lincRNA-Cox2* in macrophages. The distribution of RNA-seq reads at *lincRNA-Cox2* exon 4 region (chr1:152006173-152007782; mouse mm9 genome build) is shown for libraries prepared from either cytosolic or the nuclear RNA from mouse bone-marrow-derived macrophages stimulated with lipid A (the active component of LPS) for 2 hr or unstimulated (Ctl). These libraries were generated by Bhatt et al (*1*) and are annotated as GSM814832-C1.bam (Cytosol, Ctl), GSM814836-C5.bam (Cytosol, +LPS), GSM814837-N1.bam (Nuclear, Ctl), GSM814841-N5.bam (Nuclear, +LPS). *LincRNA-Cox2* is expressed in both the cytosolic and nuclear fractions, and induction of the lincRNA occurs in both fractions.

Fig. S13: Knockdown of hnRNP-A/B and hnRNP-A2/B1 in BMDMs. A-B, hnRNP-A/B and hnRNP-A2/B1 were knocked down using lentiviral shRNA in macrophages. Expression levels of hnRNP-A/B (A) and hnRNP-A2/B1 (B) were determined by western blotting.

Fig. S14: Expression levels of *lincRNA-Cox2* are not affected in hnRNP-A/B or hnRNP-A2/B1 knockdown cells. RNA was extracted from BMDMs stably expressing hnRNP-A/B or hnRNP-A2/B1 lentiviral shRNA and expression levels of *lincRNA-Cox2* were determined by qRT-PCR.

Fig. S15: Genes affected by *lincRNA-Cox2*, hnRNP-A/B and hnRNP-A2/B1 knockdown in macrophages. A-B,Venn diagrams representing common genes showing 2 fold induction over two RNA analysis experiments in all three cell lines either at basal levels (A) or following Pam_3CSK_4 (TLR1/2) stimulation (B). C, Table showing common genes between all three cell lines showing 2 fold induction over two separate RNA analysis experiments.

Fig. S16: Knockdown of *lincRNA-Cox2*, hnRNP-A/B or hnRNP-A2/B1 affects RNA-Poll II recruitment to *Ccl5, Irf7* and *Il-6* promoters. RNA Pol II recruitment to *Ccl5* promoter (A), *Irf7* (B) promoter and *Il6* (C) promoter were measured using Chromatin

immunoprecipiation (CHIP) on cell lines with shRNA targeting *lincRNA-Cox2*, hnRNP-A/B or hnRNP-A2/B1.

Fig. S17: Knockdown of hnRNP-A/B or A2/B1 in *lincRNA-Cox2* over-expressing cells. A-B, hnRNP-A/B (A) or hnRNP-A2/B1 (B) were knocked down in *lincRNA-Cox2* over-expressing BMDMs using lentiviral shRNA. Knockdown of hnRNP-A/B (A) and A2/B1 (B) were confirmed by western blotting.

Reference:

1. D. M. Bhatt *et al.*, Transcript dynamics of proinflammatory genes revealed by sequence analysis of subcellular RNA fractions. *Cell* 150, 279 (Jul 20, 2012).

Materials and Methods

Reagents

LPS, Pam_3CSK_4, Pam_2CSK_4, PolyI:C, DMXAA and poly(dA:dT) were from Sigma-Aldrich (St. Louis, MO). *L. monocytogenes* (clinical isolate 10403s) was from V. Boyartchuk. Customized nCounter gene expression code sets (non-enzymatic RNA profiling using bar-coded fluorescent probes) were obtained from Nanostring Technologies (Seattle, WA). Anti- hnRNP A/B, anti-hnRNPA2/B1, β-actin and USF-2 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). NFκB inhibitor (Bay 11-7083) was obtained from Tocris bioscience.

Mice and Listeria infection

C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at UMASS Medical School. Mouse strains were maintained in specific pathogen-free conditions in UMASS Medical School, and the animal protocols were carried out in accordance with the guidelines set forth by UMASS Medical School Institutional Animal Care and Use Committee.Age- and sex-matched C57BL/6 were infected with 10^5 cfu/ml *Listeria* (in 300μl) or with PBS as a control (IV via the tail vein). After 24 h spleens were harvested.

Cell Culture and stimulation

Bone marrow cells from wild-type mice were cultured in DMEM with 10% fetal bovine serum and 20% L929 supernatants to generate BMDMs or with recombinant GMCSF (20μg/ml) to generate BMDCs. BMDMs were immortalized with J2 virus. Cells were stimulated for the indicated times with LPS (TLR4) (100 ng/ml), Pam_3CSK_4 (TLR2/1) (100 nM), Pam_2CSK_4 (TLR2/6) (100 ng/ml), PolyI:C (TLR3) (25 μg/ml) or poly(dA-dT) (5 μg/ml) which was transfected with lipofectamine 2000 from Invitrogen (Grand Island, NY).

RNA interference and Plasmids

For RNA interference two separate shRNA specific to *lincRNA-Cox2* were cloned into plko vector using the following oligos: 1Forward: 5' CCGGAAGAGTAAGATTCTGAAGA TCCTCGAGGATCTTCAGAATCTTACTCTTTTTTTG 3'. 1Reverse: 5' AATTCAAAAAAA GAGTAAGATTCTGAAGATCCTCGAGGATCTTCAGAATCTTACTCTT 3'. 2Forward: 5' CCGGAAGGAATCCAGCCATCTCTCGCTCGAGCGAGAGATGGCTGGATTCCTTTTTTTG 3', 2Reverse: 5'AATTCAAAAAAAGGAATCCAGCCATCTCTCGCTCGAGCGAGAGA TGGCTGGATTCCTT 3'. 3Forward: 5' CCGGAACCTAAAGGAGGTTGACAACCTCGAG GTTGTCAACCTCCTTTAGGTTTTTTTG 3'. 3Reverse: 5' AATTCAAAAAAACCTAAAGG AGGTTGACAACCTCGAGGTTGTCAACCTCCTTTAGGTT 3'.

>*LincRNA-Cox2*
gattccctctgcgtttgcctccaagatcatgcgcgggacattctgagaaaccatgcccagagacaaaaaggaagcttggcgttgtgaaaaagccggctt
ttaatgagttgatgtgaaaagctgcacgcacattagagagacatgctgtactcttggaactttctcgacaaaagaaaacatgcataagactcctcacctct
shRNA 2
ctgcttctgcccataaaactttcctctgattca***aaggaatccagccatctctcg***ttaactcactcagccttgattcatttaatcactctaaagctctgctaagtacttgttatgt
gctgtgaatgaaactgttgctaagtacaagcaaataaacattgccactttcctaatgtggtatctaacaggcacagggtagtgaaatgagccct
shRNA 3
atggaagagctgtgggcagggc***aacctaaaggaggttgacaac***ttaatggcaagtaggagagcatttttgagagagagtgtacttaaattaccattcaa
shRNA 1
atgtaagaaaacttagactgcaaaagtgggaaggaaggtctttgggagag***aagagtaagattctgaagatc***ttgagatacaaagaaaaaaaatgaaacatttag
gataatggctagcagaaaatttgaaagtcaggagagtgaaagcttcagttttgcagtcaatcttaaatatgaatctgaatgtatatttcccttcctggaaacttttcactagc
ttccaaacacttatggaataaagaatgtatgtcagtgttctttgcctcagtttcactcatttttgtagtgagaattttggtttctttaaaaacacagaaatcttgtaagaatgctttc
attttaatcccaggtgtggaatatgaggctccttcagattgttcacagcaactgactgtgatttgcctggtgctctagcaaaggcgtgattttgccagatgcagatagcttttg
caactatgtggtgttttctgggaacttttcagaggttatatagatgatagagccctaataagtgggttgttatgtgcatattgttagttgttggctggttgctgctgtttgtggtttgtt
acatagtcacgggcaaagaagaaacaagaagaaattagatatcctgctgtctaagatcaaacttgcctcaaggaactcgatgccctaatcagcaggaagtagtcta

atgataactttatccttcccctcaattctttcttatatgatgttaaaagattgaaagaatcaaagaaaagggtgaagaagagtgggagaaagaagaacccacaaagt
agccaaaagtctggctatatatttttgaaatcaatgtgactgtctaaacatgaactgaacaaggataacagcaatagatacaccaaagcagattgggaaaagactat
gaggcctcagccctacacaaggaactaaggaatgctgagagtgggagaaatagtcctatccagggaagagcacctcagctggttatcccatacttactggccagc
cctgaaaatgtacatacaagtaacattatacaaactgagcaggttgtatttattatatatacatattacatgtgtgtatattatattatattaactttacatataatatgtattacat
ttgtagcaacaactaaattaaaaaaagaggctatgaatttgaaagagagcaaggaggggcatatagagggcttgaagggagaaaagggaagaagaaaataat
gtaattatattataatctcaac

Control shRNAs used were EV plko from Open Biosystems (Thermo Fisher Scientific, Waltham, MA) (RHS4080), a non-targeting Ctl (*1*) or GFP ctl shRNA (Thermo Fisher Scientific, Waltham, MA) (# RHS4459). shRNA to hnRNP A/B (TRCN0000103641, TRCN0000103644) and hnRNP A2/B1 (TRCN0000071138, TRCN0000071142) were obtained from open biosystems. 4µg of shRNA was transfected into HEK293T cells with packaging vectors pSpax (3µg- Addgene plasmid 12260) and pMD2 (1µg- Addgene plasmid 12259) using genejuice. After 48h media was removed and transduced into immortalized BMDMs and selected using puromycin (3mg/ml).

Full length *lincRNA-Cox2* was obtained from Genscript and subsequently cloned into pgemt and pMSCV retro vector (Clontech). mTLR1 was obtained through Prof. Medzhitov (Addgene plasmid 13080) and subcloned into pMSCV vector. Retro vectors were first transfected into HEK293Ts with packaging vectors VsVg (1µg) and Gag-pol (1µg) (both vectors are available from Clontech, Mountain View, CA) using genejuice. After 48h media was removed and transduced into immortalized BMDMs and selected using neoomycin (100µ g/ml).

RNA Extraction, Real-Time PCR, PCR, RNA Analysis, RNA-Seq

BMDM, BMDCs or splenocytes (2 to 10 × 10^6 cells/condition), were stimulated for 5 h. RNA was extracted with RNeasy kit (QIAGEN, Valencia, CA), 1µg of total or nuclear RNA was used for cDNA synthesis RNA was extracted from infected BMDMs at indicated time points using the iScript Select cDNA synthesis kit (Bio-Rad), and quantitative RT-PCR analysis was performed with the following primers: *LincRNA-Cox2F*: 5'AAGGAAGCTTGGCGTTGTGA 3' R: 5'GAGAGGTGAGGAGTCTTATG 3'. *Ptgs2/Cox2* gene F: GCTGTGGGGCAGGAAGTC 3', R:TTGGAATAGTTGCTCATCACC and β-actin F: 5'TTGAACATGGCATTGTTAC CAA3',R: 5'TGGCATAGAGGTCTTTACGGA3'. For splice variant analysis 1µg of total RNA was used for cDNA synthesis, RNA was extracted from primary BMDM or BMDC. Primers used for each confirm each variant are as follows, Variant 1 (JX682706) F:5'AAGGAAGCTTGGCGTTGTGA 3', R5' TGAAATGAGCCCTATGGAAGA 3'. Variant 2 (JX682707) F: 5'TGTCCCAGAGGGAGAAGGCAGCCTG 3', R5' TGAAATGAGCCCTA TGGAAGA 3'. Variant 3: (JX682708) F 5'CAGATGCTGGCAAGGATATGGAGAA3', R5' TGAAATGAGCCCTATGGAAGA 3'.

For RNA analysis, 100 ng of total RNA was used and hybridized to a custom designed gene codeset (non-enzymatic RNA profiling using bar-coded fluorescent probes) according to the manufacturers protocol (Nanostring technologies). The nCounter® Analysis System is an automated, multi-application, digital detection and counting system which directly profiles up to 100 molecules simultaneously from a single sample using a novel barcoding technology. Heatmaps representing differentially regulated genes were generated using R and scaled by log2(X-min(X) + 1). RNA-seq 10 mg of total RNA was used and libraries were generated using an illumina mRNA sample preparation kit (#RS-930-1001). Once samples were sized, quantified and proofed they were read on a High-Seq System (Illumina, San Diego, CA) as 100*100 single reads for BMDM experiments.

Analysis of RNA-seq data

RNA-seq 50bp reads were aligned to the mouse genome (assembly NCBIM37) using TopHat (*2*). Gene level read counts based on the resulting alignments were calculated with HTSeq

(http://www-huber.embl.de/users/anders/HTSeq/). The Ensembl64 gtf was used as the input annotation file for the program. Subsequent data analysis was performed in R and differential gene expression specific analyses were conducted with the DESeq R package (*3*). Specifically, DESeq was used to normalize gene counts, calculate fold change in gene expression, estimate pvalues and adjusted pvalues for change in gene expression values, and to perform a variance stabilized transformation on read counts to make them amenable to plotting. Using the GOstats package (*4*), the top 800 up-regulated genes (using log_2FC between base mean counts) were analyzed to detect biological processes with over-represented genes. The R package was used to color Kegg pathways according to their log_2FC values.

Circos plot

The Circos program (*5*) was used to visualize genome-wide gene expression changes. Log_2 fold-change values were computed by subtracting the log_2 variance stabilized counts of the un-stimulated sample from the log_2 variance stabilized counts of the pam3c4 stimulated sample. All protein coding genes that were annotated with particular GO IDs (GO:0006955, GO:0045088, GO:0009607, GO:0009615, GO:0006952, GO:0006954, GO:0032020, GO:0051707, GO:0051707, GO:0035455, GO:0035457, GO:0006950, GO:0001866, GO:0009611) were classified as immune genes and were colored red.

Accession numbers

All raw data is available for download from NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE40978.

ELISA

Cell culture supernatants were assayed for Ccl5 (Rantes) (RnD), IL6 (eBiosciences) and IL1β by ELISA (BD Biosciences).

RNA pull-down assay and Mass spectrometry

lincRNA-Cox2 was in vitro transcribed using RiboMAX large scale RNA production systems –T7 (sense) and Sp6 (anti-sense, Ctl) and biotinylated cytosines were incorporated during the in vitro synthesis process. The resulting products were treated with RNase-free DNase I (Promega, Madison, WI) and purified with RNeasy Mini Kit (QIAGEN). BMDMs (10×10^6 cells/condition) were separated into cytosolic and nuclear fractions using a nuclear extraction kit (Active Motif). Lysates (containing Rnasin) were incubated with 1μg of Ctl RNA for 1 h at room temperature followed by 1 h incubation with Streptavidin beads (as a pre clearing step). Lysates were then incubated with *lincRNA-Cox2* or Ctl RNA (4μg) at 4°C overnight. Interacting complexes were immunoprecipated with streptavidin beads for 3 h at room temp and visualized by silver-staining (Pierce silver stain kit, Thermo Scientific) for mass spectrometry or by immunoblotting using specific antibodies to hnRNP A/B and hnRNP A2/B1. For Mass Spectrometry analysis a specific band present in the experimental lane was extracted (corresponding region in the control lane was also extracted). Gel pieces were trypsin digested and 10% of the peptides were injected onto a C18 spray tip and eluted into the Orbitrap and analyzed with a 35 min collision induced dissociated method (UMASS proteomics core facility). The raw data was searched in mascot against the mouse subset of the swissprot database.

Cell Fixation and RNA FISH

Our standard fixation protocols have been detailed previously (*6, 7*). Briefly, cells were permeabilized in CSK buffer, 5% triton, and VRC (vanadyl ribonucleoside complex) for 1-3 min, then fixed in 4% Paraformaldehyde for 10min, and stored in 1XPBS or 70% EtOH. All DNA probes (1mg/reaction) were nick-translated using biotin-11-dUTP or digoxigenin-16-dUTP (Roche). Our standard protocols for RNA FISH and for simultaneous RNA/DNA detection have

been described previously in detail (*6, 7*). RNA-specific hybridization was carried out under non-denaturing conditions where the DNA was not accessible. Probes used for FISH were pMSCV vector expressing full-length *lincRNA-Cox2* (variant 1).

Microscopy and Image Analysis: Digital imaging analysis was performed using an Axiovert 200 or an Axiophot Zeiss microscope equipped with a 100X PlanApo objective (NA 1.4) and Chroma 83000 multi-bandpass dichroic and emission filter sets (Brattleboro, VT), set up in a wheel to prevent optical shift. Images were captured with an Orca-ER camera (Hamamatsu, NJ) or a cooled charge-coupled device (CCD) camera (200 series, Photometrics). Over 100 cells scored in each experiment. Key results were confirmed by at least two independent investigators. Images were minimally enhanced for brightness and contrast to more closely resemble what was seen by eye through the microscope (unless otherwise noted in the text/legends). Digital imaging software (developed inhouse, described below) was used to quantify signals.

Software development:
Software was developed using Matlab (using Image Processing Toolbox) to select cells and measure mean and standard deviation of cells.

1:Detect cells from blue channel

A: Estimate threshold to separate cells pixels from background.

B: Label pixels with values greater than threshold as belonging to a cell - pixels less than the threshold are labeled as background.

C:Discard connected cell regions with less that 10000 pixels and greater than 30000 pixels (to remove cells undergoing division).

D:Discard cell regions located at the edge of the image (not fully contained by the image).

E:A pixel mask of each valid cell is ready to be used in step 2.

2:In the green channel, measure the Adjusted Mean and Standard Deviation for each valid cell

a: Calculate the adjusted mean for each cell.

i. Calculate the background value of the green channel for the current image - the background value the most frequent value of background pixels in the green channel.

ii: Calculate the mean for each cell, subtracting the background value for that image (calculated in i).

b: Calculate the variance of each cell.

i. Calculate the variance of green chanel pixels for each cell.

3: Calculate normalized histograms and cumulative distributions for the adjusted mean and standard deviation of Ctl (128 cells total) and lincRNA-Cox2 shRNA cells (187 cells total).

Chromatin immunoprecipitation (ChIP)
Immortalized BMDMs expressing either control shRNA, or shRNA targeted against lincRNA-Cox2, hnRNP-A2B or hnRNP-AB were treated with the media control or the TLR2 agonist Pam_3CSK_4 for 5 hr, and were subjected to ChIP assays using the ChIP-IT express chromatin immunoprecipitation kit (Active Motif, Carlsbad, CA) following the manufacturer's instruction. Briefly, 10 million cells were fixed in 1% formaldehyde for 10 min at 37°C to cross-link the DNA/protein complexes, followed by sonication of the isolated nuclear pellet using Bioruptor

UCD-200 (Diagenode Inc., Sparta, NJ) to shear the DNA. An aliquot of the chromatin sample (50µl) was reverse cross-linked overnight, treated with RNAse A and Proteinase K, followed by phenol:chloroform extraction of the DNA to confirm DNA shearing to 200-500 bp chromatin fragments by agarose gel electrophoresis, and the DNA quantitation by Nanodrop. Sheared chromatin (5µg/IP) was immunoprecipitated with 2µg anti-RNA pol II (Active Motif; Clone 4H8) or IgG1 isotype control (Imgenex) antibody, and 20µl magentic beads for 4 hr. After reversal of the cross-linking and protein digestion with proteinase K, immunoprecipitated DNA was purified with the PCR purification kit (Qiagen). Quantitative real-time PCR analysis was performed on the input DNA (diluted 1:10) and the ChIP samples using iQ SYBR Green Supermix reagent (Bio-Rad) using using the following parameters (50°C for 2 min, 95°C for 2 min, followed by 45 cycles of 95°C for 15 s, 60°C for 30 sec and 72°C for 45 sec, followed by melting curve analysis). The primer sequences used for detecting the RNA polII recruitment on the murine genes are- *Ccl5* (for: 5'- GCCAACCCATCTTGATACCC -3', and rev: 5'- ACAGTGATGACAGGGTAGCC -3'), *IRF7* (for: 5'- AAGGGCAGTGAAGAGAAGCT -3', and rev: 5'- CCTACCAGTGCAGTCCTCAA -3'), *IL6* (for: 5'- AGACTTCCATCCAGTTGCCT -3', and rev: 5'- CAGGTCTGTTGGGAGTGGTA -3'), *gapdh* (for: 5'- TAGGACTGGATAAGCAGGGC -3', and rev: 5'-GAACAGGGAGGAGCAGAGAG -3'). All oligo sequences were designed using Primer3web (http://primer3.wi.mit.edu/), and were targeted within the 500 nucleotides region downstream of the transcription start site of each gene. The specificity of amplification was assessed for each target gene by melting curve analysis, and the amplicon size was confirmed by agarose gel electrophoresis. RNA polII enrichment in the IgG and RNA polII ChIP samples were normalized to the input DNA, and is represented as % Input. Experiments were performed at least three times with independent chromatin samples and yielded similar results.

Sucrose sedimentation analysis

Macrophages were seeded at 5 million cells in a 100mm dish. After 16 h of culture cells were pretreated with cycloheximide (100 μg.ml^{-1}) for 10 min at 37ºC or with harringtonine (2µg.ml^{-1}) for 25 min followed by cycloheximide (100 μg.ml^{-1}) for 10 min. Cells were washed twice in ice-cold PBS+Cycloheximide (100 μg.ml^{-1}) and scraped in 1ml of PBS+Cycloheximide (100 μg.ml^{-1}). Cells were then pelleted at 500g for 5 min at 4ºC and lysed in 1ml of lysis buffer (10mM Tris-HCl pH. 7.5; 5mM MgCl2; 100mM KCl; 1% Triton X-100; 2mM DTT; 100 μg/ml Cycloheximide and 1X Protease-Inhibitor Cocktail EDTA-free (Roche). Lysate was homogenized by gently pipetting up and down for a total of eight strokes using a P1000 pipettor. After incubation at 4ºC for 10 min, lysate was cleared at 1300.g for 10 min at 4ºC, the supernatant recovered and absorbance at 260nm measured. 10 A260 units complemented or not with 35 mM of EDTA were loaded on top of a 10 to 50% (Weight/Volume) sucrose gradient (20mM HEPES-KOH pH. 7.4; 5mM MgCl2; 100mM KCl; 2mM DTT; 100 μg.ml^{-1} of Cycloheximide) and centrifuged in a SW-40ti rotor at 35,000 rpm for 2h40min at 4ºC. After centrifugation, samples were collected from the top of the gradient into 15 fractions while absorbance at 254nm was measured. Collected fractions were complemented with 0.4 femto moles of a firefly coding RNA (Spike-In), SDS (1% final), proteinase K (200 μg.ml^{-}1) and incubated at 42ºC for 45 min. After proteinase K treatment, RNA was extracted using one volume of Phenol (pH 4.5):Chloroform:Iso-amyl alcohol (25:24:1). The recovered aqueous phase was supplemented with 20 μg of glycogen, 300 mM sodium acetate pH 5.2, and 10 mM $MgCl_2$. RNA was precipitated with 3 volumes of 100% ethanol at −20°C overnight. After a wash with 70% ethanol, RNA was re-suspended in 50 μl of water.

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