Supplementary Information for

*C*is-acting lnc-Cxcl2 restrains neutrophil-mediated lung inflammation by inhibiting epithelial cell CXCL2 expression in virus infection

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Supplementary text

Materials and Methods

Plasmids, reagents and virus

cDNA fragments of La were amplified from MLE-12 cells cDNA, and then were cloned into pcDNA3.1 vector.

BAY 11-7082，BAY 11-7085, QNZ (EVP4593) were from Selleck. ELISA kits detecting mouse CXCL2 and CCL2 were from R&D Systems. Anti-La (D19B3), Anti-P65 (D14E12), Anti-P50 (D4P4D), Anti-STAT1 (D1K9Y), Anti-STAT3 (124H6), Anti-c-JUN (60A8), Anti-EZH2 (D2C9), Anti-Flag (D6W5B), Anti-Lamin A/C (4C11), Anti-H3K4me3 (C42D8), Anti-CBP (D6C5), Anti-Rpb1 CTD (4H8), Anti-P-Rpb1 CTD (Ser2) (E1Z3G) and Normal rabbit IgG antibodies were from Cell Signaling Technology. Anti-GAPDH (3H12) and Anti-Actin (6D1) antibodies were from MBL. Anti-Gr-1 (EPR22909-135) antibody was from Abcam. PE anti-mouse CD45 (30-F11), APC anti-mouse Ep-CAM (G8.8), PerCP/Cy5.5 anti-mouse CD11c (N418), FITC anti-mouse Siglec-F (S17007L), PE/Cy7 anti-mouse NK1.1 (PK136), APC anti-mouse CD11b (M1/70), FITC anti-mouse Gr-1 (RB6-8C5), FITC anti-mouse I-A/I-E (M5/114.15.2) antibodies were from BioLegend. APC CD4 Monoclonal Antibody (RM4-5), APC CD19 Monoclonal Antibody (eBio1D3 (1D3)) and PE F4/80 Monoclonal Antibody (BM8) were from Thermo Scientific. PE Rat Anti-Mouse CD8a (53-6.7) antibody was from BD Pharmingen.

GFP-VSV and VSV were amplified using HEK293T cells. Influenza virus strain A/Puerto Rico/8/1981 H1N1 (PR8) was amplified using VERO cells in VP-SFM

(Thermo Scientific) supplemented with 4 mM glutamine and 1 μg/mL TPCK-treated trypsin (Sigma-Aldrich). Viral titers were determined by plaque assay.

Mouse primary alveolar epithelial cells, resident alveolar macrophages and trachea epithelial cells isolation

Mouse primary alveolar epithelial cells (AECs), resident alveolar macrophages (AMs) and trachea epithelial cells (TECs) were isolated according to the published protocol (1, 2) with some modifications. Briefly, mouse lung was digested using 50 U/mL Dispase (Roche) at room temperature for more than 45 min, then cells were collected for antibody labeling and cell sorting. $FSC^{hi}SSC^{hi}$, singlet, $CD45^{-}EPCAM^{+}$ cells were sorted as AECs, $CD45^{+}CD11c^{+}Siglec\text{-}F^{+}$ cells were sorted as AMs. Mouse tracheas were separated and cut along the vertical axis, then were digested in 0.15% Pronase (Roche) overnight at 4 ℃. On the second day, cells were collected and incubated in plates at 37 ℃ for 5 h, after that the suspended cells were collected as TECs.

Quantitative PCR

Total RNA was extracted using Trizol reagent (Thermo Scientific), cDNA was synthesized using ReverTra Ace qPCR RT Master Mix (TOYOBO). For lnc-Cxcl2 detection, RNA was treated with DNase I and extracted again to remove genome contamination, oligo(dT) was used to synthesize cDNA.

SYBR Green Realtime PCR Master Mix (TOYOBO) was used for quantitative PCR (q-PCR). Each sample data was normalized to its RPL32 expression. Primers used

for q-PCR analysis were listed in *SI Appendix*, Table S5.

Transcriptome analysis

lnc-Cxcl2$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells or siRNA-transfected A549 cells were infected with VSV or IAV for 12 h. Then mRNAs from these cells were purified and the libraries were generated. RNA-seq was performed on Illumina platform. Obtained clean reads were aligned to the reference genome using Hisat2 v2.0.5, and featureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene. $|\log_2\text{Fold Change}| > 1$ and ajusted *p* value (padj) < 0.05 were used to define differentially expressed genes. The sequencing data were deposited in NCBI GEO under accession code GSE156949 and GSE179951.

DNase I sensitivity assay

Wild-type, lnc-Cxcl2-deficient, or La-deficient MLE-12 cells were uninfected or infected with VSV for 12 h, then the cells were collected and treated with DNase I for 30 min at 37 ℃. Genomic DNA was extracted from these cells and quantified by q-PCR. Genomic DNA from cells not treated with DNase I was also extracted and used as the input control. Primers used to detect different promoter regions by q-PCR analysis were listed in *SI Appendix*, Table S5.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) analysis was performed using SimpleChIP

Enzymatic Chromatin IP Kit (Cell Signaling Technology) according to the manufacturer's instructions. Briefly, cells were cross-linked with 1% formaldehyde followed by micrococcal nuclease digestion. 10 μg digested chromatin was used as one sample and incubated with antibody overnight at 4 °C, then protein G magnetic beads were added for another two hours' incubation. After washing for four times, chromatin DNA was eluted from beads and quantified by q-PCR. Primers used to detect different promoter regions by q-PCR analysis were listed in *SI Appendix*, Table S5.

RNA pull-down assay

Biotin-labeled lnc-Cxcl2 or lnc-CXCL2-4-1 was obtained by *in vitro* transcription and was crosslinked with M280 Streptavidin magnetic beads (Thermo Scientific) for 15 min at room temperature. MLE-12 or A549 cells were lysed using IP lysis buffer (Thermo Scientific), and then the protein lysate was added to the RNA-bound beads and incubated in protein-RNA binding buffer (20 mM Tris-HCl at pH 7.5, 50 mM NaCl, 2 mM $MgCl_2$, 0.1% Tween-20 Detergent) for 1 h at 4 °C. After incubation, beads were washed four times with wash buffer (20 mM Tris-HCl at pH 7.5, 10 mM NaCl, 0.1% Tween-20 Detergent), captured proteins were isolated by heating in SDS loading buffer and detected by immunoblot analysis. For MS analysis, nuclear proteins of MLE-12 cells were used in pull-down assay.

RNAi

siRNAs were transfected into MLE-12 cells or A549 cells using Lipofectamine

RNAiMAX Reagent (Thermo Scientific) according to the manufacturer's instructions. After transfection for 24 h, cells were infected with VSV or IAV for indicated hours followed by RNA extraction for cytokine expression analysis or DNase I treatment for chromatin accessibility analysis. For silencing each mRNA, a mixture of three siRNAs was used, for silencing each lncRNA, a mixture of three siRNAs and three ASOs was used. The target sequences of siRNAs were listed in *SI Appendix*, Table S6.

RNA immunoprecipitation

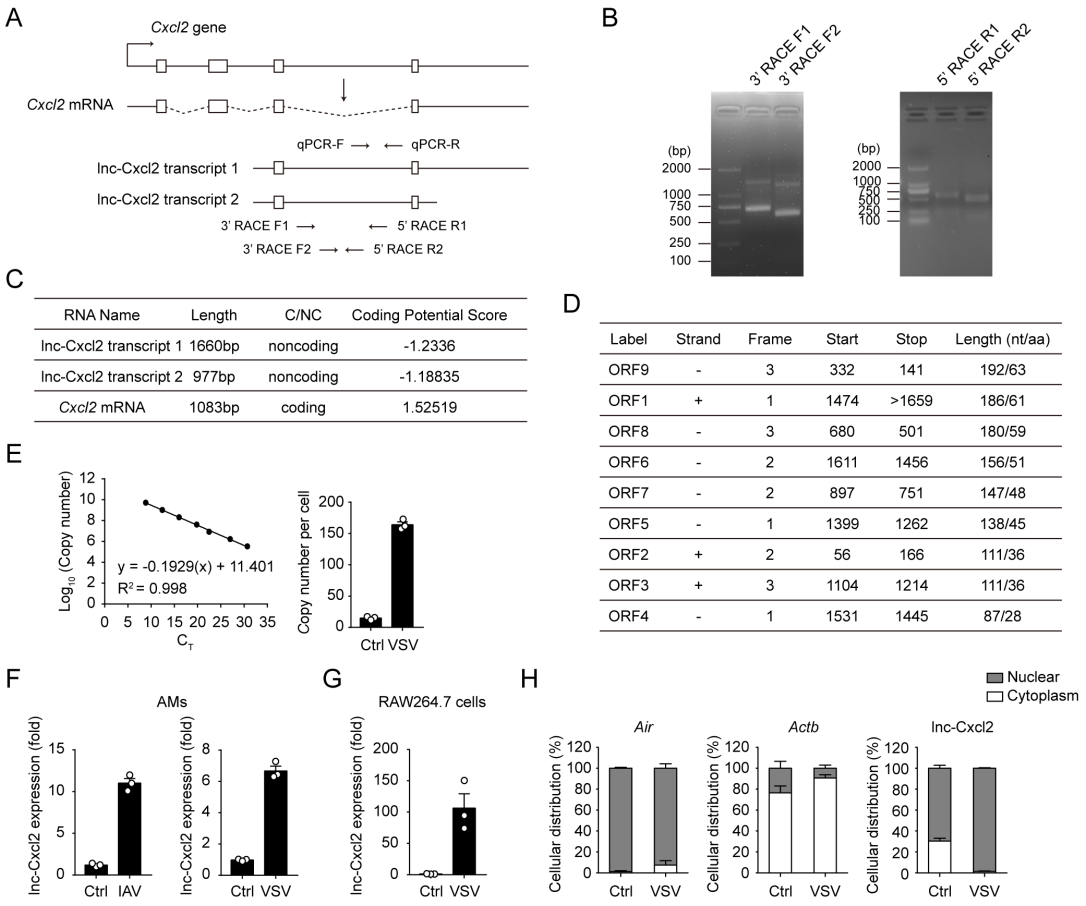
MLE-12 cells were lysed in lysis buffer (10mM HEPES-NaOH at pH 7.5, 100 mM KCl, 5 mM $MgCl_2$ and 1% NP-40), and the supernatants were collected and incubated with antibodies overnight. Dynabeads Protein G (Thermo Scientific) were washed with NT2 buffer (50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$ and 0.05% NP-40) and added into supernatants for 2 h' incubation. Then the beads were washed five times with NT2 buffer, RNA was eluted using Proteinase K and isolated for q-PCR analysis. Primers used for q-PCR analysis were listed in *SI Appendix*, Table S5.

SI References

1. H. C. Lam, A. M. Choi, S. W. Ryter, Isolation of mouse respiratory epithelial cells and exposure to experimental cigarette smoke at air liquid interface. *J. Vis. Exp.*, 2513 (2011).

2. M. Sinha, C. A. Lowell, Isolation of highly pure primary mouse alveolar epithelial type II cells by flow cytometric cell sorting. *Bio. Protoc.* **6**, e2013 (2016).

Supplementary Figures



RNA Name	Length	C/NC	Coding Potential Score
lnc-Cxcl2 transcript 1	1660bp	noncoding	-1.2336
lnc-Cxcl2 transcript 2	977bp	noncoding	-1.18835
Cxcl2 mRNA	1083bp	coding	1.52519

Label	Strand	Frame	Start	Stop	Length (nt/aa)
ORF9	-	3	332	141	192/63
ORF1	+	1	1474	>1659	186/61
ORF8	-	3	680	501	180/59
ORF6	-	2	1611	1456	156/51
ORF7	-	2	897	751	147/48
ORF5	-	1	1399	1262	138/45
ORF2	+	2	56	166	111/36
ORF3	+	3	1104	1214	111/36
ORF4	-	1	1531	1445	87/28

Fig. S1. lnc-Cxcl2 expression is increased in the nucleus of mouse lung epithelial cells in response to viral infection.

(*A*) Schematic illustration of genomic locations of lnc-Cxcl2 transcripts and primers used to detect lnc-Cxcl2. (*B*) RACE analysis of lnc-Cxcl2 in MLE-12 cells infected with VSV (MOI = 1) for 12 h. (*C*) Coding potential analysis of lnc-Cxcl2 using Coding Potential Calculator. (*D*) Coding potential analysis of lnc-Cxcl2 using NCBI Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/orffinder/). (*E*) Standard curve generated using plasmid expressing lnc-Cxcl2 (left), and q-PCR analysis of the copy number of lnc-Cxcl2 in MLE-12 cells infected with VSV (MOI = 1) for 12 h (right).

(*F*) q-PCR analysis of lnc-Cxcl2 expression in AMs infected with IAV (MOI = 1) or VSV (MOI = 1) for 12 h. (*G*) q-PCR analysis of lnc-Cxcl2 expression in RAW264.7 cells infected with VSV (MOI = 1) for 6 h. (*H*) q-PCR analysis of *Air*, *Actb* mRNAs and lnc-Cxcl2 expressions in nucleus and cytoplasm of MLE-12 cells infected with VSV (MOI = 1) for 12 h. Data are representative of three independent experiments with $n = 3$ biological replicates (*E*-*G*; shown as mean + SEM) or from three independent experiments (*H*; shown as mean + SEM).

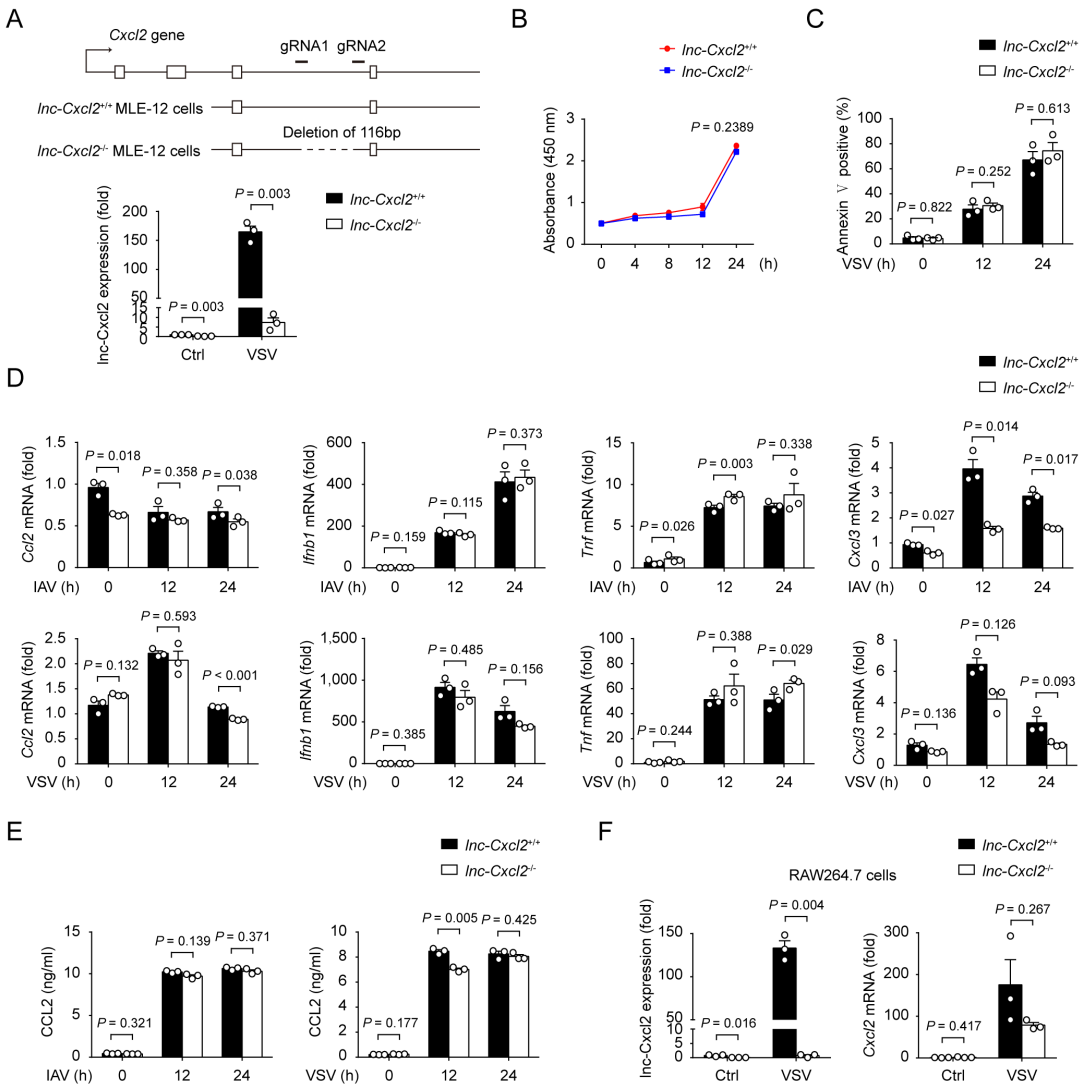


Fig. S2. lnc-Cxcl2 selectively inhibits *Cxcl2* expression in lung epithelial cells during viral infection.

(*A*) Schematic illustration of the generation of *lnc-Cxcl2*$^{-/-}$ MLE-12 cells (top), q-PCR analysis of lnc-Cxcl2 expression in *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells infected with VSV (MOI = 1) for 12 h (bottom). (*B*) CCK-8 (Cell counting kit) assay of cell proliferation of *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells within indicated hours. (*C*) Flow cytometric analysis of cell death of *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells infected with VSV (MOI = 1) for indicated hours. (*D*) q-PCR analysis of *Ccl2*, *Ifnb1*,

Tnf and *Cxcl3* mRNA expressions in *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells infected with IAV (MOI = 1) or VSV (MOI = 1) for indicated hours. (*E*) ELISA of CCL2 level in the supernatant of *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells infected with IAV (MOI = 1) or VSV (MOI = 1) for indicated hours. (*F*) q-PCR analysis of lnc-Cxcl2 and *Cxcl2* mRNA expressions in *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ RAW264.7 cells infected with VSV (MOI = 1) for 6 h. Data are representative of three independent experiments with $n = 3$ biological replicates (*C-F*; shown as mean + SEM), two-tailed unpaired Student's *t*-test, or three independent experiments with $n = 6$ biological replicates (*B*; shown as mean ± SEM), two-way ANOVA analysis.

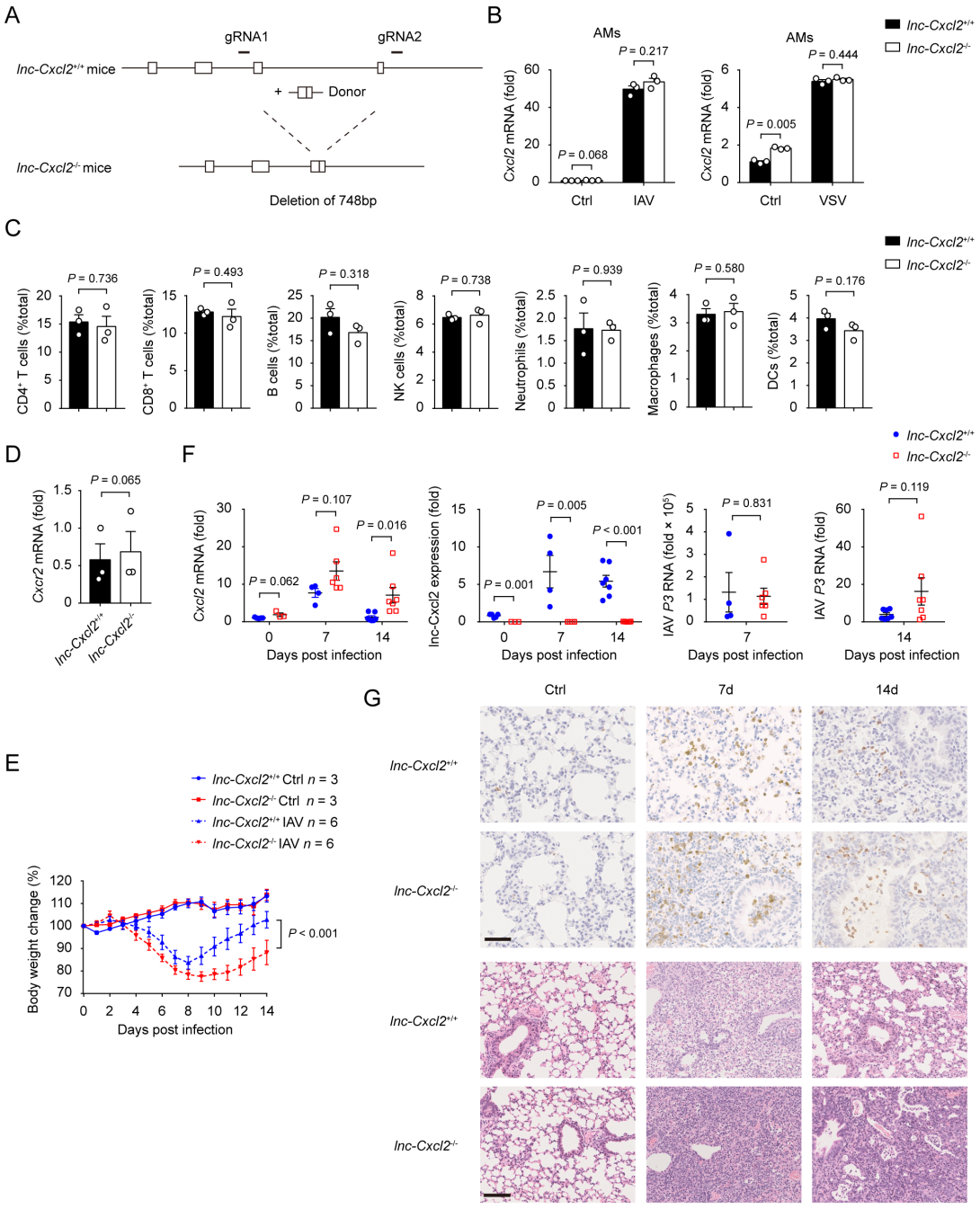


Fig. S3. lnc-Cxcl2 restrains influenza virus-induced lung inflammation *in vivo*.

(*A*) Schematic illustration of the generation of *lnc-Cxcl2*$^{-/-}$ mice. (*B*) q-PCR analysis of *Cxcl2* mRNA expression in AMs from *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ mice infected with IAV (MOI = 1) or VSV (MOI = 1) for 12 h. (*C*) Flow cytometric analysis of the propagations of $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, $NK1.1^+$ NK cells,

$CD11b^+Gr\text{-}1^+$ neutrophils, $CD11b^+F4/80^+$ macrophages, and $CD11c^+MHC\,II^+$ DCs in the spleen from *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ mice. (*D*) q-PCR analysis of *Cxcr2* mRNA expression in bone marrow neutrophils from *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ mice. (*E*) Body weight change analysis of *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ mice intranasally infected with IAV (50 PFU) for two weeks. (*F*) q-PCR analysis of *Cxcl2* mRNA and lnc-Cxcl2 expressions and IAV load indicated by virus polymerase P3 expression in lungs from *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ mice intranasally infected with IAV (50 PFU) for indicated times. (*G*) Immunohistochemistry staining of $Gr\text{-}1^+$ neutrophils (top) and hematoxylin- and eosin staining (bottom) of lung sections from mice described in (*F*). Scale bar, 50 μm (top) or 100 μm (bottom). Data are representative of three independent experiments with $n = 3$ biological replicates (*B-D*; shown as mean + SEM), from two independent experiments with 3 or 6 mice per group (*E*; shown as mean ± SEM) or pooled from four independent experiments with 3 to 7 mice per group (*F* and *G*; shown as mean + SEM in *F*), two-tailed unpaired Student's *t*-test (*B-D*, *F*), two-way ANOVA analysis (*E*).

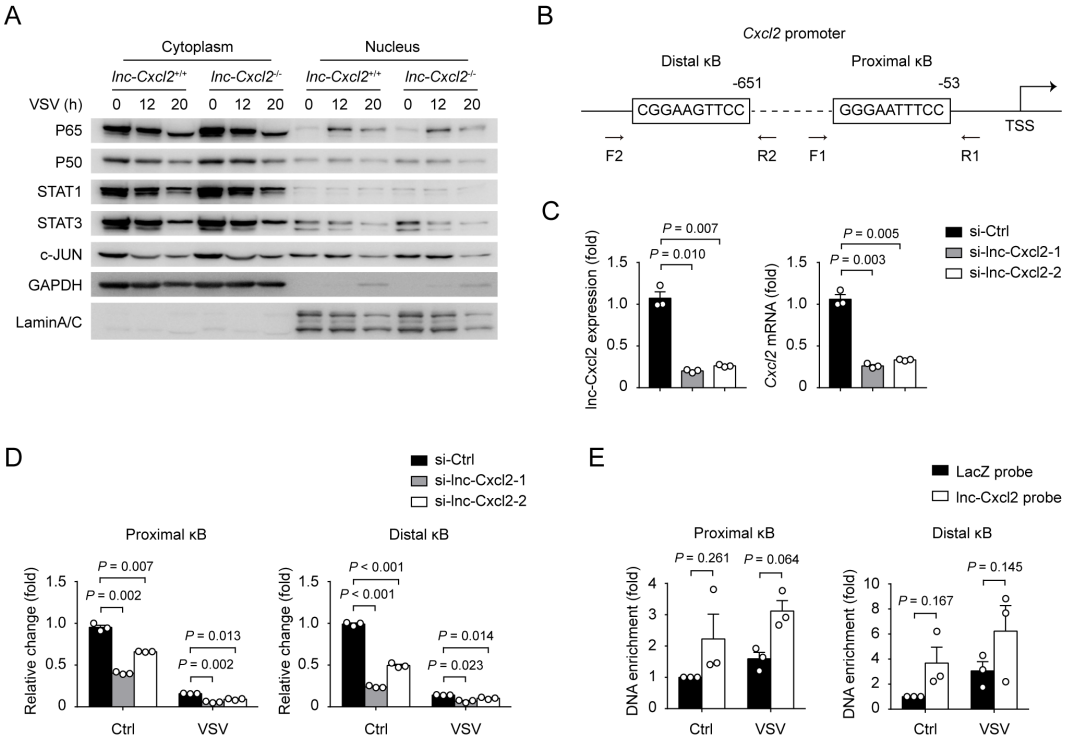


Fig. S4. lnc-Cxcl2 binds to and maintains the repressed chromatin state of *Cxcl2* promoter *in cis*.

(*A*) Immunoblot analysis of the indicated transcription factors in the cytoplasm and nucleus of *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells infected with VSV (MOI = 1) for indicated hours. (*B*) Schematic illustration of two κB sites and primer locations in *Cxcl2* promoter. (*C*) q-PCR analysis of lnc-Cxcl2 and *Cxcl2* mRNA expressions in MLE-12 cells transfected with negative ctrl siRNA or lnc-Cxcl2 siRNA. (*D*) q-PCR analysis of the DNase I sensitivity of *Cxcl2* promoters in MLE-12 cells transfected with negative ctrl siRNA or lnc-Cxcl2 siRNA followed by VSV infection (MOI = 1) for 12 h. (*E*) q-PCR analysis of enrichment level of *Ccl2* promoter that purified by lnc-Cxcl2 probes in MLE-12 cells infected with VSV (MOI = 1) for 12 h. Data are representative of three independent experiments (*A*), three independent experiments with $n = 3$ biological

replicates (*C* and *D*; shown as mean + SEM) or from three independent experiments (*E*; shown as mean + SEM), two-tailed unpaired Student's *t*-test.

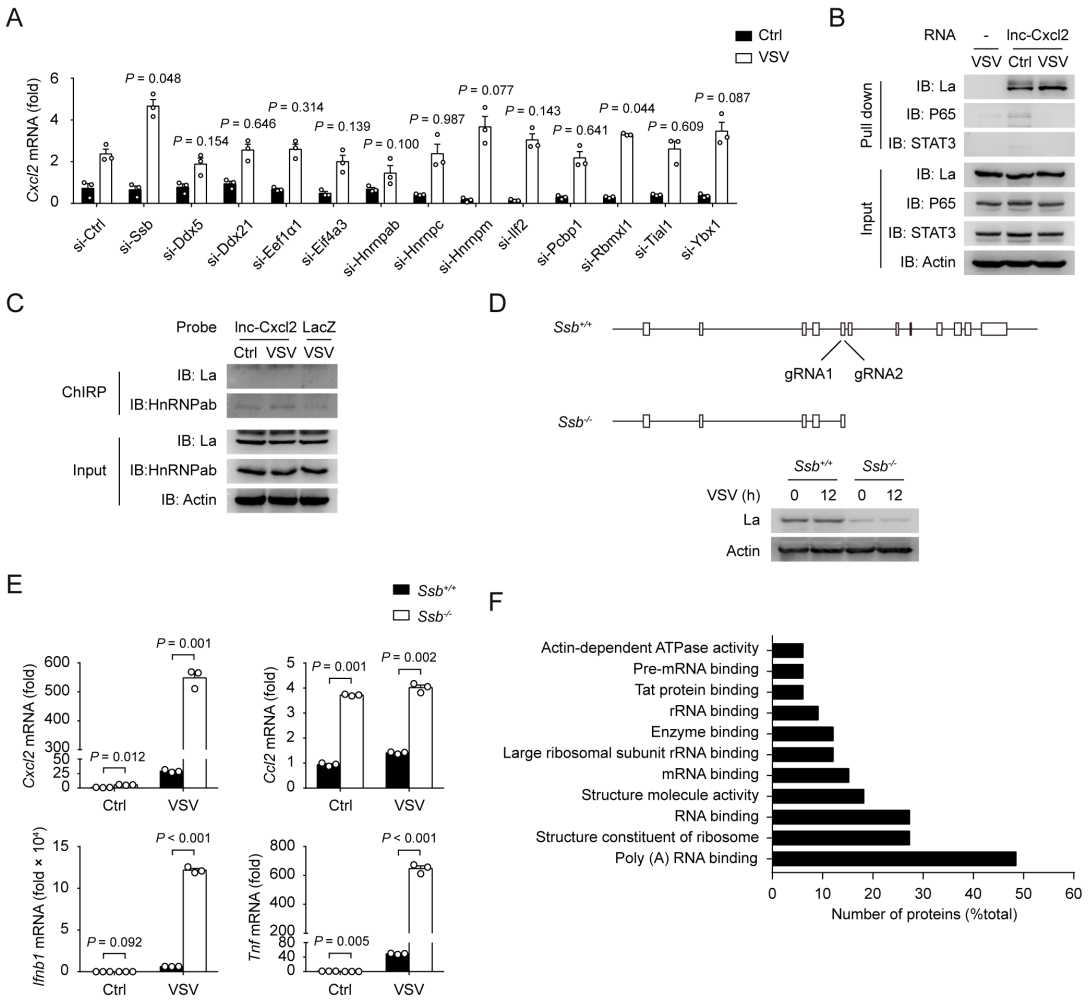


Fig. S5. lnc-Cxcl2 inhibits *Cxcl2* expression through ribonucleoprotein La.

(*A*) q-PCR analysis of the *Cxcl2* mRNA expression in MLE-12 cells transfected with negative ctrl siRNA or siRNAs targeting indicated mRNAs (50 nM) followed by VSV infection (MOI = 1) for 12 h. (*B*) Immunoblot analysis of lnc-Cxcl2 and La interaction in MLE-12 cells infected with VSV (MOI = 1) for 12 h. (*C*) Immunoblot analysis of proteins purified by lnc-Cxcl2 probes in RAW264.7 cells infected with VSV (MOI = 1) for 6 h. (*D*) Schematic illustration of the generation of *Ssb*$^{-/-}$ MLE-12 cells (top) and immunoblot analysis of La protein level in *Ssb*$^{+/+}$ and *Ssb*$^{-/-}$ MLE-12 cells infected with VSV (MOI = 1) for 12 h (bottom). (*E*) q-PCR analysis of *Cxcl2*, *Ccl2*, *Ifnb1*, and *Tnf*

mRNA expressions in *Ssb*$^{+/+}$ and *Ssb*$^{-/-}$ MLE-12 cells infected with VSV (MOI = 1) for 12 h. (*F*) Gene ontology analysis of the molecular functions of La-interacting proteins. *P* value < 0.05. Data are representative of three independent experiments (*B* and *C*) or three independent experiments with $n = 3$ biological replicates (*A* and *E*; shown as mean + SEM), two-tailed unpaired Student's *t*-test.

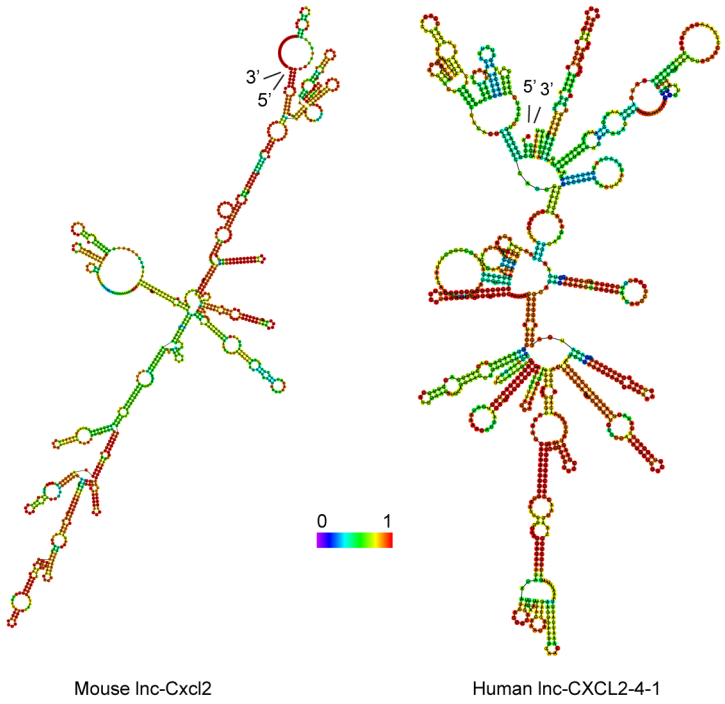


Fig. S6. Secondary structure of mouse lnc-Cxcl2 and human lnc-CXCL2-4-1.

Secondary structure prediction of mouse lnc-Cxcl2 and human lnc-CXCL2-4-1 by RNAfold web server (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi). The minimum free energy structures are colored by base-pairing probabilities.

Supplementary Tables

Table S1. Sequence of lnc-Cxcl2 determined by RACE.

lnc-Cxcl2 transcript 1 (5'-3')
TGCCCAGACCCTCCTGCCGCCCCATGCTCACCCTAAAGAAACCTCGTGCCTTATA
ATGATCCCCACATCTCTCTGCAGAGCCACTCTCAAGGGCGGTCAAAAAGTTTGC
CTTGACCCTGAAGCCCCCCTGGTTCAGAAAATCATCCAAAAGATACTGAACAAG
TGAGTCGCGATTTGTGCTTACACCTGACTGGCACCCGATTTCTGAAAATTATAGC
AGGGAAAATCAAGGTTAACTTCAGTTCAGGAACCTGAATTATTATTTTCATTGTT
AACTTTGTCCTGTGAGCATCAGTTTGAATTGATAGAAGGGTACGACTAGCTCCTT
TCATCTACACACGTAAAAGTCCTTCCTTAATGGATGGTCGCTGTGTGTCCCTAAG
AATTAAGTAGATAGAACCAACTAGCGCTGGTGTAAAGCCTGTTATCTATTAAGAT
TCAATACTTTACCCCTGCCCTTTCCATGTCTGTGGGCTGTAAAGTCTAACTCTTG
GTTTTGGTCAAGGTGAGCCTCTAAGGCTTCCCGATGAAGAAGAGCTGGGGTTTC
CAGTGAGAGGCTTGAAATTCAGGTGTAGGCTCTCGCTCTTATCCTGACTGAGTT
CAGTGAAAAAGCAATAGGTATTGTTCTTGTTTACAACGTACGCGAAGTCATGCCT
TGACATTTTTACTCTTAACATCATTTGGAAAGGTTCAAGCTGGTACAATTGTAAC
GCCAGCTCTACTATTCTAGTTTCAAAGCGCCCAGAGTGTTTATTGCTGGAAAGTC
CAGCAATAGGTAGCAGGTCCCTTGTGGAGGCTAGAGATGGTTTCGTTTGGAGAG
TAAACCAAGGTCCAGGATGAAGTCAAAAGAAGGGGGCTCTGCAACCCGCCTTT
AAGATACCCACTAACAGCAACCATTTTCTCATCACAGAGGCAAGGCTAACTGAC
CTGGAAAGGAGGAGCCTGGGCTGCTGTCCCTCAACGGAAGAACCAAAGAGAA
AGAAAAAAACAAACAGCACCCGGGAAGCCTGGATCGTACCTGATGTGCCTCGC
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CCCAGATGTTGTTATGTTTATTTTGATATTTAAAGATATGCATTCGCTAATTCACTG
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GTTCAATTAAAGTTATTTAACTTATATAGTTGGAAGGTGATAAATTTTTAAACCTA
TTTATTCATTAGTTTCTGGGGAGAGGGTGAGTTGGGAACTAGCTACATCCCACCC
ACACAGTGAAAGAGACTGGGGATAAGGGGTGGGGGTGGGGACAAATAGATGCA
GTCGGATGGCTTTCATGGAAGGAGTGTGCATGTTCACATCATTTTTTTGTAAGCA
CCGAGGAGAGTAGAACAGCTGTTATTTAGGTTTCAGTGTTTGTAAACTGTATGTA
CAACATTTTTGATGCTGGATTTCAATGTAATGTTGTGAGTAACCCTTGGACATTTT
ATGTCTTCCTCGTAAGGCACAGTGCCTTGCTTAGCAATTGTTTTGTCATGCCTTTT
CGTGTCTTGAAGTGGACACATTTATTTATTCATGTATTTTTACAAATAACAAAAAA
TAAAAACGTCTGTTAGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
lnc-Cxcl2 transcript 2 (5'-3')
TGCCCAGACCCTCCTGCCGCCCCATGCTCACCCTAAAGAAACCTCGTGCCTTATA
ATGATCCCCACATCTCTCTGCAGAGCCACTCTCAAGGGCGGTCAAAAAGTTTGC

CTTGACCCTGAAGCCCCCCTGGTTCAGAAAATCATCCAAAAGATACTGAACAAG
TGAGTCGCGATTTGTGCTTACACCTGACTGGCACCCGATTTCTGAAAATTATAGC
AGGGAAAATCAAGGTTAACTTCAGTTCAGGAACCTGAATTATTATTTTCATTGTT
AACTTTGTCCTGTGAGCATCAGTTTGAATTGATAGAAGGGTACGACTAGCTCCTT
TCATCTACACACGTAAAAGTCCTTCCTTAATGGATGGTCGCTGTGTGTCCCTAAG
AATTAAGTAGATAGAACCAACTAGCGCTGGTGTAAAGCCTGTTATCTATTAAGAT
TCAATACTTTACCCCTGCCCTTTCCATGTCTGTGGGCTGTAAAGTCTAACTCTTG
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GCCAGCTCTACTATTCTAGTTTCAAAGCGCCCAGAGTGTTTATTGCTGGAAAGTC
CAGCAATAGGTAGCAGGTCCCTTGTGGAGGCTAGAGATGGTTTCGTTTGGAGAG
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AAGATACCCACTAACAGCAACCATTTTCTCATCACAGAGGCAAGGCTAACTGAC
CTGGAAAGGAGGAGCCTGGGCTGCTGTCCCTCAACGGAAGAACCAAAGAGAA
AAAAAAAAAAAAAAAAAAAAA

Table S2. Differentially expressed genes between *lnc-Cxcl2*$^{+/+}$ and *lnc-Cxcl2*$^{-/-}$ MLE-12 cells after VSV infection.

Gene_ID	Gene_Name	log_2Fold Change	padj
ENSMUSG00000069045	Ddx3y	-12.0178	1.19E-25
ENSMUSG00000056673	Kdm5d	-10.6469	1.10E-16
ENSMUSG00000068457	Uty	-10.0624	2.70E-13
ENSMUSG00000026574	Dpt	-5.2229	9.47E-07
ENSMUSG00000057219	Armc7	2.772075	2.22E-05
ENSMUSG00000042244	Pglyrp3	3.825828	2.74E-05
ENSMUSG00000097352	C920009B18Rik	8.317647	5.03E-05
ENSMUSG00000024561	Mbd1	-3.1801	0.000219
ENSMUSG00000024810	Il33	-2.29913	0.000324
ENSMUSG00000019982	Myb	-3.61385	0.00033
ENSMUSG00000087107	AI662270	3.204905	0.000391
ENSMUSG00000021745	Ptprg	-4.53405	0.000391
ENSMUSG00000062896	Rpl31-ps11	-4.16322	0.007209
ENSMUSG00000041559	Fmod	-2.02468	0.007662
ENSMUSG00000023484	Prph	3.322772	0.009482
ENSMUSG00000036545	Adamts2	2.197305	0.009831
ENSMUSG00000090957	Gm7535	-2.7301	0.018716
ENSMUSG00000067780	Pi15	-2.97685	0.018716
ENSMUSG00000025150	Cbr2	7.388866	0.024055
ENSMUSG00000058427	Cxcl2	2.488358	0.032147
ENSMUSG00000056025	Clca3a1	-3.93459	0.032147

Table S3. lnc-Cxcl2-interacting proteins identified by MS analysis in MLE-12 cells.

Gene Symbol	Sample 1			Sample 2		
	Score	Matches	Coverage	Score	Matches	Coverage
Hnrnpab	608	44(23)	26%	559	54(23)	28%
Ddx21	372	57(24)	33%	29	3(1)	5%
Rbmxl1	327	25(12)	20%	176	23(8)	20%
Ilf2	319	30(17)	21%	345	31(17)	15%
Eef1a1	172	11(6)	9%	57	9(3)	11%
Hnrnpc	74	10(4)	20%	223	23(11)	24%
Tial1	72	3(2)	7%	41	3(2)	7%
Hnrnpm	58	6(1)	5%	39	2(1)	3%
Ssb	46	10(3)	23%	132	13(6)	16%
Ybx1	42	5(1)	10%	48	9(2)	23%
Ddx5	40	5(2)	6%	37	7(1)	9%
Eif4a3	34	7(1)	9%	63	8(4)	10%
Pcbp1	27	5(1)	10%	71	8(3)	13%

Table S4. La-interacting proteins identified by MS analysis in MLE-12 cells.

Sample	0 h			12 h		
Gene Symbol	Score	Matches	Coverage	Score	Matches	Coverage
Myh9	3997	159(102)	46%	1869	94(46)	37%
Actb	503	31(17)	42%	398	28(15)	47%
Ssb	391	20(11)	28%	387	20(11)	26%
Myl6	380	16(11)	41%	176	10(6)	30%
Myl12a	355	10(8)	46%	170	6(5)	34%
Krt6b	230	12(12)	9%	188	9(6)	8%
Krt90	225	10(8)	8%	153	6(4)	6%
Tpm1	223	8(7)	19%	72	3(2)	11%
Krt78	201	3(3)	2%	237	5(4)	5%
Rplp2	183	5(4)	69%	160	6(4)	69%
Rpl12	149	5(3)	40%	235	6(3)	49%
Gm5478	130	10(4)	7%	139	10(3)	6%
Gemin5	117	6(2)	4%	45	4(0)	2%
Pabpc1	105	6(3)	11%	207	13(8)	21%
Gapdh	105	4(2)	15%	53	3(1)	12%
Krt2	90	7(3)	4%	81	7(2)	6%
Krt42	82	4(3)	13%	99	5(2)	17%
Gm49450	75	1(1)	5%	61	1(1)	5%
Alb	67	1(1)	2%	59	1(1)	2%
Eef1a1	61	5(1)	12%	70	5(1)	4%
2210010C04Rik	59	26(2)	8%	90	23(2)	8%
Ddx5	55	4(2)	8%	88	8(3)	15%
Rpl30	50	2(1)	37%	66	3(1)	37%
Rpl4	47	5(0)	19%	30	3(0)	11%
Rpl23	47	1(1)	33%	82	1(1)	33%
Ptbp1	42	3(1)	9%	77	2(1)	5%
Rplp1	41	1(1)	14%	52	1(1)	14%
Rpl6	37	2(1)	3%	40	2(1)	6%
Mybbp1a	36	4(1)	2%	98	10(2)	8%
Mettl3	35	3(0)	1%	27	4(0)	1%
Gm8797	34	2(0)	20%	34	2(1)	32%
Rpl27	28	3(0)	22%	34	4(1)	36%
Npm1	25	1(0)	9%	69	1(1)	4%
Fam98a	25	1(0)	2%	26	1(0)	2%

Rps11	21	2(0)	6%	23	2(0)	18%

Table S5. Primers used for q-PCR analysis in this study.

Name	Forward (5'-3')	Reverse (5'-3')
lnc-Cxcl2	CAACGTACGCGAAGTCAT	ATAAACACTCTGGGCGCT
Rpl32	TTAAGCGAAACTGGCGGAAAC	TTGTTGCTCCCATAACCGATG
Cxcl2	CAGAAGTCATAGCCACTCTCAA	CTCCTTTCCAGGTCAGTTAGC
Cxcl2 pre-mRNA	TTCCTCGGGCACTCCAGACT	AAACATCAGCCTCGAATCCG
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ifnb1	ATGAGTGGTGGTTGCAGGC	TGACCTTTCAAATGCAGTAGATTCA
Tnf	AAGCCTGTAGCCCACGTCGTA	GGCACCACTAGTTGGTTGTCTTTG
Cxcl3	CAGAAGTCATAGCCACTCTC	TGGACTTGCCGCTCTTCAGT
Ssb	AAGAATGGCTAGACGATAAAGGC	GCTTGTGTCTTCCTTCATGCT
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Air	ACTTTGACAGAACAATCGGCT	GAACATTTGCAAAGGACAGT
IAV P3	GGCCGACTACACTCTCGATGA	TGTCTTATGGTGAATGACCTGGTTT
RPL32	GCCCAAGATCGTCAAAAAGAGA	TCCGCCAGTTACGCTTAATTT
lnc-CXCL2-1-1	AGGCTGGAATGTAGTGGTGC	CTGGCCACAATGGTTCATGC
lnc-CXCL2-2-1	CTTGCCTTGCTTGTGTGCTT	TCTCATACTGGGACAGGGCT
lnc-CXCL2-4-1	TCCCAAGTAACCACCAGTTGT	TGTGCCACCATTTCCTCATC
CXCL2	CTGCCAGTGCTTGCAGAC	CTGGTCAGTTGGATTTGCCA
CXCL8	CTGATTTCTGCAGCTCTGTG	TTCACTGGCATCTTCACTG
SSB	TGGATAGACTTCGTCAGAGGAG	TCTTTGGCTTTACCCAATGCTT
Cxcl2 proximal	CAGTAGAATGAGGCAG	GAGAGCTCCTTTTATGC

κB site		
Cxcl2 distal κB site	CACCAAGTCTCTGTTTC	GGAGATGTGTCAAAAGC
Ccl2 proximal κB site	TTACTGGGGGTCCTTTCCCA	GGAGTGGCTCTGCTTTCACT
Ccl2 distal κB site	ATGTGAGAGCGCCACTCTTT	TGGTAGCTCTCTGCCCTGTT

Table S6. Target sequences of siRNAs used in this study.

siRNA	Target sequence (5'-3')
ASO-m-lnc-Cxcl2-1_001	TCATTTGGAAAGGTTCAAGC
ASO-m-lnc-Cxcl2-1_002	TCTGCAACCCGCCTTTAAGA
ASO-m-lnc-Cxcl2-1_003	AAGATACCCACTAACAGCAA
si-m-lnc-Cxcl2-1_001	GGTCCAGGATGAAGTCAAA
si-m-lnc-Cxcl2-1_002	GGTTCAAGCTGGTACAATT
si-m-lnc-Cxcl2-1_003	GGTTTCGTTTGGAGAGTAA
ASO-m-lnc-Cxcl2-2_001	CTGGCACCCGATTTCTGAAA
ASO-m-lnc-Cxcl2-2_002	CACGTAAAAGTCCTTCCTTA
ASO-m-lnc-Cxcl2-2_003	TCCAGTGAGAGGCTTGAAAT
si-m-lnc-Cxcl2-2_001	TCAGGAACCTGAATTATTA
si-m-lnc-Cxcl2-2_002	CCATGTCTGTGGGCTGTAA
si-m-lnc-Cxcl2-2_003	GCATCAGTTTGAATTGATA
si-m-Ssb_001	AGACGATAAAGGCCAAATA
si-m-Ssb_002	GAAAGTACTAGAAGGACAT
si-m-Ssb_003	GAGAAGAACCCGCATCAAA
si-m-Ddx5_001	GCTTGTCGCTTGAAGTCTA
si-m-Ddx5_002	GGTTCTAAATGAATTCAAA
si-m-Ddx5_003	GGTCGAAGACAGAGGTTCA
si-m-Ddx21_001	GAAAGAAGCAAGCGGAGAT
si-m-Ddx21_002	TCAGTTACGCTTGGAAAGA
si-m-Ddx21_003	TCACAGAAATACAGGAGAA
si-m-Eef1a1_001	ACCAGCAAATACTATGTGA
si-m-Eef1a1_002	TCAGAAGAGATACGAGGAA
si-m-Eef1a1_003	AGAAGAGATACGAGGAAAT
si-m-Eif4a3_001	CGTGCTATCAAGCAGATAA
si-m-Eif4a3_002	GGATATCCAGGTTCGAGAA
si-m-Eif4a3_003	CTGATGAAATGTTGAACAA
si-m-Hnrnpab_001	CCCAACACTGGACGATCAA
si-m-Hnrnpab_002	GGAAGATCCTGTGAAGAAA
si-m-Hnrnpab_003	GGTTGTTGACTGTACAATA
si-m-Hnrnpc_001	GGTGTGAAACGATCTGCAG
si-m-Hnrnpc_002	GTGGATTCTCTTCTGGAAA
si-m-Hnrnpc_003	GGATGATGACGATAATGAA
si-m-Hnrnpm_001	CCGAGAGATTGATGTTCGA
si-m-Hnrnpm_002	GCTGTGCAAGCAATATCTA

si-m-Hnrnpm_003	GCACAGTATTTGTAGCAAA
si-m-Ilf2_001	GCTTCTCAGTCCACAGTTA
si-m-Ilf2_002	GGCAGGTAGGATCATATAA
si-m-Ilf2_003	CAGCTATCTTGCTTCTGAA
si-m-Pcbp1_001	CTACCAATGCCATCTTTAA
si-m-Pcbp1_002	CTCACTTTGCCATGATGCA
si-m-Pcbp1_003	AACTTAATCGGCTGCATAA
si-m-Rbmxl1_001	AGATGCAGCTAGAGATATG
si-m-Rbmxl1_002	AGCAGAGATTACCCAAGCT
si-m-Rbmxl1_003	TACCGTGATTACAGTCATT
si-m-Tial1_001	ACAAGCAATGACCCATATT
si-m-Tial1_002	CTGCATTAGCTGCTATGAA
si-m-Tial1_003	CAACAGTATGGACAGTATA
si-m-Ybx1_001	GGCGAAGGTTCCCACCTTA
si-m-Ybx1_002	GGTAGACCAGTGAGACAGA
si-m-Ybx1_003	GGTGCAGGAGAGCAAGGTA
ASO-h-lnc-CXCL2-4-1_001	TCTTCCTTACAGTAGAACCC
ASO-h-lnc-CXCL2-4-1_002	GTGTTCCTGAATTCTTACCA
ASO-h-lnc-CXCL2-4-1_003	GAGGAACTTCTTTCGAAGTA
si-h-lnc-CXCL2-4-1_001	TCTGCCAAATGCTGTTCTT
si-h-lnc-CXCL2-4-1_002	GTAACATGCTCAAGGTGCT
si-h-lnc-CXCL2-4-1_003	GCACAACTGGTGTAATTCT
si-h-SSB-1	GACCAACAAGAATCCCTAA
si-h-SSB-2	CGGGTGATTTAGATGATCA