

Supplementary Figures

CRISPR-Cas9 mediated functional dissection of 3'-UTRs

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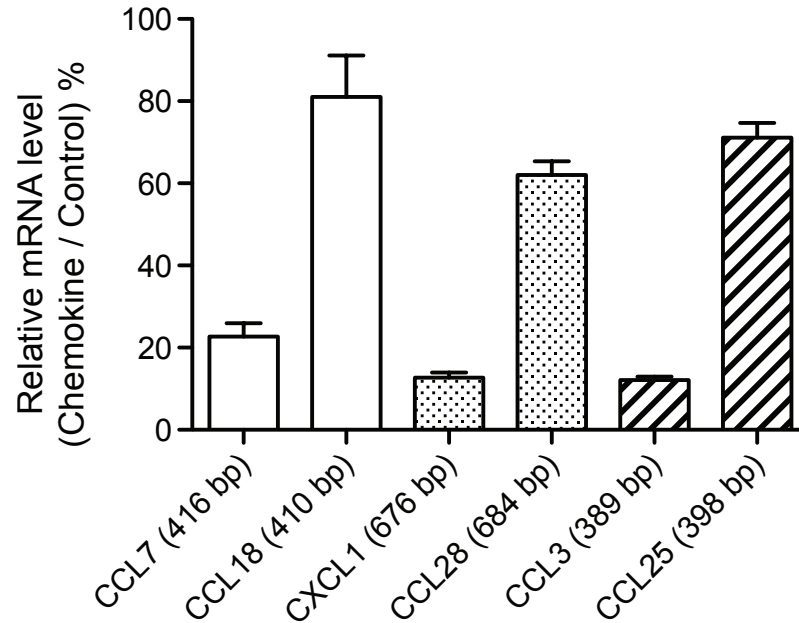


Figure S1: 3'-UTR length is not the primary determinant of functional activity in the reporter assay. 3'-UTRs were cloned into the BTV reporter and steady state mRNA levels were measured as in Figure 1 A and B. Lengths of each 3' UTR DNA segment are shown in parentheses. Three different fills (blank, dots, and slash) represent different groups of 3'-UTRs with a similar length.

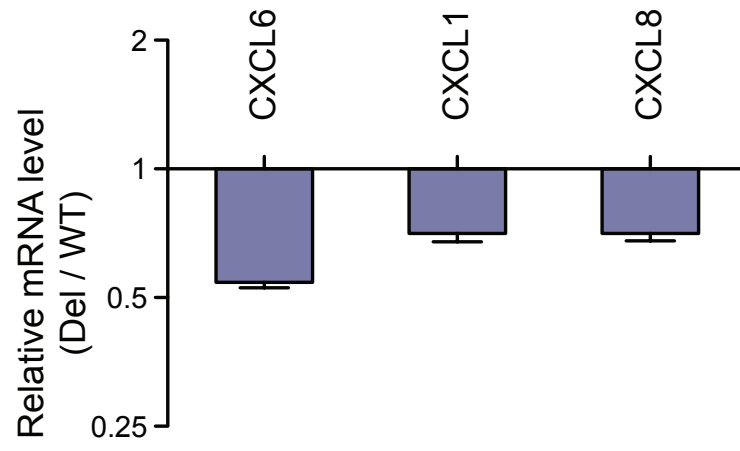


Figure S2. Evaluation of the effects of targeting *CXCL1*, *CXCL6*, and *CXCL8* 3'-UTRs with independent pairs of gRNAs. Experiments were performed as described in Fig. 1E. gRNA positions are provided in Supplementary Data.

CCL3_det_F1	GTGAGGAGTGGGTCCAGAAA
ddect_CC4-F1	CCAAACCAAAGAAGCAAGC
ddect_CC7-F1	AGGCTGATGGGCTAGACAGA
ddect_CC17-F1/2	CCTGTGTGACAGCAGCAACT
ddect_CC25-F1/2	GGCCTCTCTCATTGCTTCTG
ddect_CC26-F1	GTGGCAAGGCCAGAAGACTA

CCL3_det_R1	aaaagcgctcagtaggagga
ddect_CC4-R1	cggtgcaacagtccttagtt
ddect_CC7-R1	acaatgtgagcccaacctct
ddect_CC17-R1	gaaccctcccagagtgacag
ddect_CC25-R1	ccacagccctggaatctaag
ddect_CC26-R1	agaattocgtatcggggtct

CCL3
CCL4
CCL7
CCL17
CCL25
CCL26

IV. Primers for detection of chemokine mRNA level with qRT-PCR (Related to Figure 1E)

Forward primer Name	Forward primer sequences
CXCL1_F4	CCCAAGAACATCCAAGTGTG
CXCL3_F1	gcaggaattcactcaaga
CXCL6_F1	gcttgagtttctgcccagtc
CXCL7_F3	CAGCAACTCACCTCACTCA
CXCL8_F4	AAGAAACCACCGAAGGAAC
CXCL10_F3	AGGAACCTCCAGTCTCAGCA
CXCL11_F3	GCAGCAAAGCTGAAGTAGCA
CXCL17_F5	AGCGTCACCTCACCTGTCT
CCL3_F2	gcagcagacagtggtcagtc
CCL4_F5	ACACAGCTGGGTCTGAAG
CCL7_F3	GGCTGAGACCAAACAGAAA
CCL25_F4	CACACCCAAGGTGCTTTGA
CCL26_F4	GTGGGAGTGACATATCCAAGAC

Reverse primer Name	Reverse primer sequences
CXCL1_R4	TGGATTGTCACTGTTACAGCA
CXCL3_R1	gggtgcccccttgttcagta
CXCL6_R1	gggaggtcatagtggtcaa
CXCL7_R3	GTTTGTCTTGTGGGAGGA
CXCL8_R4	AATTTCTGTGTTGGCGCAGT
CXCL10_R3	TGATCTCAACACGTGGACAAA
CXCL11_R3	ATGCAAAGACAGCGTCTCT
CXCL17_R5	TGTCGGTGCAGCTGTAAGTT
CCL3_R2	agcagcaagtgatgcagaga
CCL4_R5	CTTCCTCGCGGTGTAAGAAA
CCL7_R3	CTGTAGCAGCAGGTAGTTGAAG
CCL25_R4	CACACCTTCTGTGTCCTTG
CCL26_R4	CCTTGGATGGGTACAGACTTTT

Gene symbol
CXCL1
CXCL3
CXCL6
CXCL7
CXCL8
CXCL10
CXCL11
CXCL17
CCL3
CCL4
CCL7
CCL25
CCL26

V. The primers for detection of chemokine transcription in 4sU labeling experiments with qRT-PCR (Related to Figure 2C,D)

1. PCR from the exon/intron

Forward primer Name	Forward primer sequences
X1_E.I-F3	CTCTTCCGCTCCTCTCACAG
X6_E.I-F2	ccagcaacctgcccataaaa
X8_E.I-F1	CAAGAGCCAGGAAGAAACCA
b-actin-F-3	cttccagcagatgtggatca

Reverse primer Name	Reverse primer sequences
X1_E.I-R3	actgactgagcgggctgtc
X6_E.I-R2	ACTTCCACCTTGGAGCACTG
X8_E.I-R1	ggaaaacgctgtaggtcagaa
b-actin-R-3	aaagccatgccaatctcatc

Gene symbol
CXCL1
CXCL6
CXCL8
beta-actin

2. PCR from exon only

Forward primer Name	Forward primer sequences
X1_exon-F2	ACCTCCTCGCCAGCTCTTC
CXCL6_F1	gcttgagtttctgcccagtc
X8_exon-F2	GAGCACTCCATAAGGCACAA
b-actin-F-3	cttccagcagatgtggatca

Reverse primer Name	Reverse primer sequences
X1_exon-R2	GCTACCAGGAGCAGGAGCA
CXCL6_R1	gggaggtcatagtggtcaa
X8_exon-R2	AGCTGCAGAAATCAGGAGG
b-actin-R-3	aaagccatgccaatctcatc

Gene symbol
CXCL1
CXCL6
CXCL8
beta-actin

VI. The primers for cloning 3'UTRs in the enhancer reporter pLS-mp (Related to Figure 3)

1. Cloning full length chemokine 3'UTRs in pLS-mp

Primers used for inserting 3' UTR sequences in a forward orientation

Forward primer Name	Forward primer sequences
Eh-Fw_X1_F	ATCGTCTAGAcagaaggaggaggaagctcac
Eh-Fw_X3_F	ATCGTCTAGAgatcattgacacttctgcccaggtg
Eh-Fw_X6_F	ATCGTCTAGAccatgcatcataaaaattgcccagtc
Eh-Fw_X8_F	ATCGTCTAGAcagaatcagtggaatgcccagtg
Eh-Fw_X10_F	ATCGTCTAGAAACAGAGGGGAGCAAAATCGATGC
Eh-Fw_C3_F	ATCGTCTAGATTCGAGGCCAGCGACCTC
Eh-Fw_C4_F	ATCGTCTAGACAGGAAGTCTTCAGGGAAGGTCAC
Eh-Fw_C7_F	ATCGTCTAGAGACTGAACTGAAACAAGCCATGAC

Reverse primer Name	Reverse primer sequences
Eh-Fw_X1_R	GTAACCTGCAGGcccccttgttcttaagccagaaacac
Eh-Fw_X3_R	GTAACCTGCAGGtgataaattctcttttccaagggaaagag
Eh-Fw_X6_R	GTAACCTGCAGGctccaaatgacaatacaagtaaaaaaac
Eh-Fw_X8_R	GTAACCTGCAGGccatacaaatcagaacagtaaaaaaatttgg
Eh-Fw_X10_R	GTAACCTGCAGGAACCTTTTGTATCTTTCAACATTTAGATAGT
Eh-Fw_C3_R	GTAACCTGCAGGACAGCCCTGAACAAAAGCATCCGAT
Eh-Fw_C4_R	GTAACCTGCAGGTGAAAACACAGAAATCAAATGTTATCC
Eh-Fw_C7_R	GTAACCTGCAGGTGAAAATTTGGGAGTCATACATATGCAAAAT

Gene symbol
CXCL1
CXCL3
CXCL6
CXCL8
CXCL10
CCL3
CCL4
CCL7

Primers used for inserting 3' UTR sequences in a reverse orientation

Forward primer Name	Forward primer sequences
Eh-Rv_X1_F	GTAACCTGCAGGcagaaggaggaggaagctcac
Eh-Rv_X3_F	GTAACCTGCAGGgtatcattgacacttctgcccaggtg
Eh-Rv_X6_F	GTAACCTGCAGGccatgcatcataaaaattgcccagtc
Eh-Rv_X8_F	GTAACCTGCAGGccaagaatcagtggaatgcccagtg
Eh-Rv_X10_F	GTAACCTGCAGGAACAGAGGGGAGCAAAATCGATGC

Reverse primer Name	Reverse primer sequences
Eh-Rv_X1_R	ATCGTCTAGAccccttgttcttaagccagaaacac
Eh-Rv_X3_R	ATCGTCTAGAtgataaattctcttttccaagggaaagag
Eh-Rv_X6_R	ATCGTCTAGActccaaatgacaatacaagtaaaaaaac
Eh-Rv_X8_R	ATCGTCTAGAccatacaaatcagaacagtaaaaaaatttgg
Eh-Rv_X10_R	ATCGTCTAGAAACCTTTTGTATCTTTCAACATTTAGATAGT

Gene symbol
CXCL1
CXCL3
CXCL6
CXCL8
CXCL10

Eh-Rv_C3_F GTAACCTGCAGGTTGAGGCCAGCGACCTC
 Eh-Rv_C4_F GTAACCTGCAGGCAGGAAGTCTTCAGGGAAGGTCCAC
 Eh-Rv_C7_F GTAACCTGCAGGACTGAACTGAAAACAAGCCATGAC

Eh-Rv_C3_R ATCGTCTAGAACAGCCCTGAACAAAAGCATCCGAT
 Eh-Rv_C4_R ATCGTCTAGATGAAAACACACAGAATCAAATGTGTTATCC
 Eh-Rv_C7_R ATCGTCTAGATGAAAATTTGGGAGTCATACATATGCAAAT

CCL3
 CCL4
 CCL7

VII. The oligonucleotide pool for expressing sgRNAs targeting CXCL1 3'UTR (Related to Figure 5A, B)

1. Synthesis of oligo pool: Each oligonucleotide consists of two common regions and a specific gRNA sequence (XXXXXXXXXXXXXXXXXXXX) as shown below:

TATGCCCTGTGCTGAGCTTGGAAATTAATACGACTCACTATAXXXXXXXXXXXXXXXXXXXXGTTTATGAGCTAGAAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTG

Serial_ID	sgRNA sequence contained (XXXXXXXXXXXXXXXXXXXX) *	Modification of gRNA
CXCL1.IVT.sgRNA_1	ggtgagcttctcctcctccttc	+G
CXCL1.IVT.sgRNA_2	ggaaggaggagggaagctcac	+G
CXCL1.IVT.sgRNA_3	gggaggagggaagctcactgg	NA
CXCL1.IVT.sgRNA_4	ggataagggcaggcctccttc	+2G
CXCL1.IVT.sgRNA_5	ggtcactggtggetgttctga	+2G
CXCL1.IVT.sgRNA_6	ggtggctgttctcctgaagg	-2G
CXCL1.IVT.sgRNA_7	ggcttctgttctcctataaggca	+2G
CXCL1.IVT.sgRNA_8	ggtcttctgttctcctataaggcc	+2G
CXCL1.IVT.sgRNA_9	ggttctctctctgttctcctataa	+2G
CXCL1.IVT.sgRNA_10	ggttctctctctgttctcctata	+2G
CXCL1.IVT.sgRNA_11	ggaaggaggccctgcccttat	+G
CXCL1.IVT.sgRNA_12	ggcccttatagggaacagaag	re1G
CXCL1.IVT.sgRNA_13	ggaagagagacacagctgcag	+2G
CXCL1.IVT.sgRNA_14	ggcacattaggcacaatccagg	+2G
CXCL1.IVT.sgRNA_15	ggaacacattaggcacaatcc	+2G
CXCL1.IVT.sgRNA_16	ggacacagctgcagaggccacc	+2G
CXCL1.IVT.sgRNA_17	ggaagcgtgctcaaacacatt	+2G
CXCL1.IVT.sgRNA_18	ggaatgtgtttgagcatcgctt	+2G
CXCL1.IVT.sgRNA_19	ggattctatgtaataatttt	re1G
CXCL1.IVT.sgRNA_20	ggttttaggtgtaaaataatta	+2G
CXCL1.IVT.sgRNA_21	ggttttaggtgtaaaataattaa	+2G
CXCL1.IVT.sgRNA_22	ggaatataataggacagtgtgc	+2G
CXCL1.IVT.sgRNA_23	ggtcaaaaagaatgaatataat	+2G
CXCL1.IVT.sgRNA_24	ggatccagattgaactaacttg	+2G
CXCL1.IVT.sgRNA_25	ggaatccagattgaactaactt	+2G
CXCL1.IVT.sgRNA_26	ggaatccagattgaactaact	+G
CXCL1.IVT.sgRNA_27	ggaacccaagttagtccaatc	+2G
CXCL1.IVT.sgRNA_28	ggattcatatattaattga	-G
CXCL1.IVT.sgRNA_29	ggaaatattaacataatgac	re1G
CXCL1.IVT.sgRNA_30	ggtcattatgtaataatctctg	+2G
CXCL1.IVT.sgRNA_31	ggcacagtggctggcatgttg	+2G
CXCL1.IVT.sgRNA_32	ggccagcctctatcacagtggc	+2G
CXCL1.IVT.sgRNA_33	ggtccgccagcctctatcacag	+2G
CXCL1.IVT.sgRNA_34	ggcatgccagcactgtgatag	+2G
CXCL1.IVT.sgRNA_35	ggccagcactgtgatagaggc	+2G
CXCL1.IVT.sgRNA_36	ggccactgtgatagaggctgg	+G
CXCL1.IVT.sgRNA_37	ggatctcatgtggcatttgct	+G
CXCL1.IVT.sgRNA_38	ggctggcggatccaagcaaa	NA
CXCL1.IVT.sgRNA_39	ggccttcacaatgatctcat	re1G
CXCL1.IVT.sgRNA_40	ggccaatgagatcattgtga	NA
CXCL1.IVT.sgRNA_41	ggaatgagatcattgtgaaggc	+2G
CXCL1.IVT.sgRNA_42	ggatgagatcattgtgaaggca	+2G
CXCL1.IVT.sgRNA_43	ggagatcattgtgaaggcag	re1G
CXCL1.IVT.sgRNA_44	ggaaatgatttcacagtgtg	re1G
CXCL1.IVT.sgRNA_45	ggaagacataaaatgtccaa	+G
CXCL1.IVT.sgRNA_46	ggaagacataaaatgtcca	re1G
CXCL1.IVT.sgRNA_47	ggaaaatgttctaataatccct	+2G
CXCL1.IVT.sgRNA_48	ggcattttatgtctttcttcta	+2G
CXCL1.IVT.sgRNA_49	ggtaaaactaccattaaaca	re1G
CXCL1.IVT.sgRNA_50	ggcatactgccttgtttaa	-G

CXCL1.IVT.sgRNA_51	ggtagttttacagtgtttc	-G
CXCL1.IVT.sgRNA_52	ggtgtttctggcttagaacia	+G
CXCL1.IVT.sgRNA_53	ggtttctggcttagaacia	re1G
CXCL1.IVT.sgRNA_54	ggtttctggcttagaaciaag	+G
CXCL1.IVT.sgRNA_55	ggaaaaactcgtttgatttt	+G
CXCL1.IVT.sgRNA_56	ggaaaaactcgtttgattttt	+2G
CXCL1.IVT.sgRNA_57	ggaaaaactcgtttgatttttg	+2G
CXCL1.IVT.sgRNA_58	ggaaaaactcgtttgatttttgg	+2G
CXCL1.IVT.sgRNA_59	ggaaactcgtttgatttttggg	+2G
CXCL1.IVT.sgRNA_60	ggttgatttttgggggaaaca	+2G
CXCL1.IVT.sgRNA_61	ggatttttgggggaaacia	re1G
CXCL1.IVT.sgRNA_62	ggatcaccagattttccagtaa	+2G
CXCL1.IVT.sgRNA_63	ggaaacaagggtacctttac	+G
CXCL1.IVT.sgRNA_64	ggctacctttactggaaaatc	+G

* Note: gRNAs were modified to begin with two Gs as needed. +G, one additional G was added at the 5' end;
+2G, two additional Gs were added at the 5' end;
re1G, the first nucleotide at the 5' end was replaced with G;
-G, the first nucleotide at the 5' end was removed;
NA, no modification was made with the gRNA.

2. Primers for PCR amplification of the oligonucleotide pool: (Related to Figure 5)

Forward primer Name	Forward primer sequences	Reverse primer Name	Reverse primer sequences
IVT.lib_CXCL1	tatgcctgttgctgagcttg	IVT.lib_R	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGC
CXCL1.P1_F20	acagtgacaaatccaactga	CXCL1.P3_R20	accagatttccagtaaagg
CXCL1full_Nx_F	cctactgggaagaagttgagagtggatgggtccaatggacagtgacaaatccaactga	CXCL1full_Nx_R	gacagactcgttgacagctgtacgtaacgctactcaggcaaccagatttccagtaaagg

VIII. sgRNA sequences targeting CXCL3 N1N2 and PCR primers for sequencing library (Related to Figure 4)

sgRNA sequences targeting CXCL3 3'-UTR DNA

CXCL3_outside N1N2	ATTGAAATGCAAGCAATTAG
CXCL3_outside N1N2	GCAATTAGTGGATCACTGTT
CXCL3_outside N1N2	AGTGGATCACTGTTAGGGTA
CXCL3_outside N1N2	TTCTGCAGCGTTTCTCTTTC
CXCL3_outside N1N2	TGCAGCGTTTCTCTTCCCT
CXCL3_inside N1N2	TTAGACATTTTATGTC TTGC

For First Round PCR:

fX3crNv-F1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGACAGGGTGGGAACTGGAGGG
fX3crNv-F2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGTTCAACATTTTATGCTGAAG
fX3crNv-R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCNNNNgtacaaatgtaacagtAATGATAAATTCTC

For Second Round PCR:

D2nd-F	AATGATACGGCGACCACCGAGATCTACACNNNNNNNACACTCTTCCCTACACGACG
D2nd-R	CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTACTGGAGTTCAGACGTGT