

SUPPLEMENTAL MATERIAL

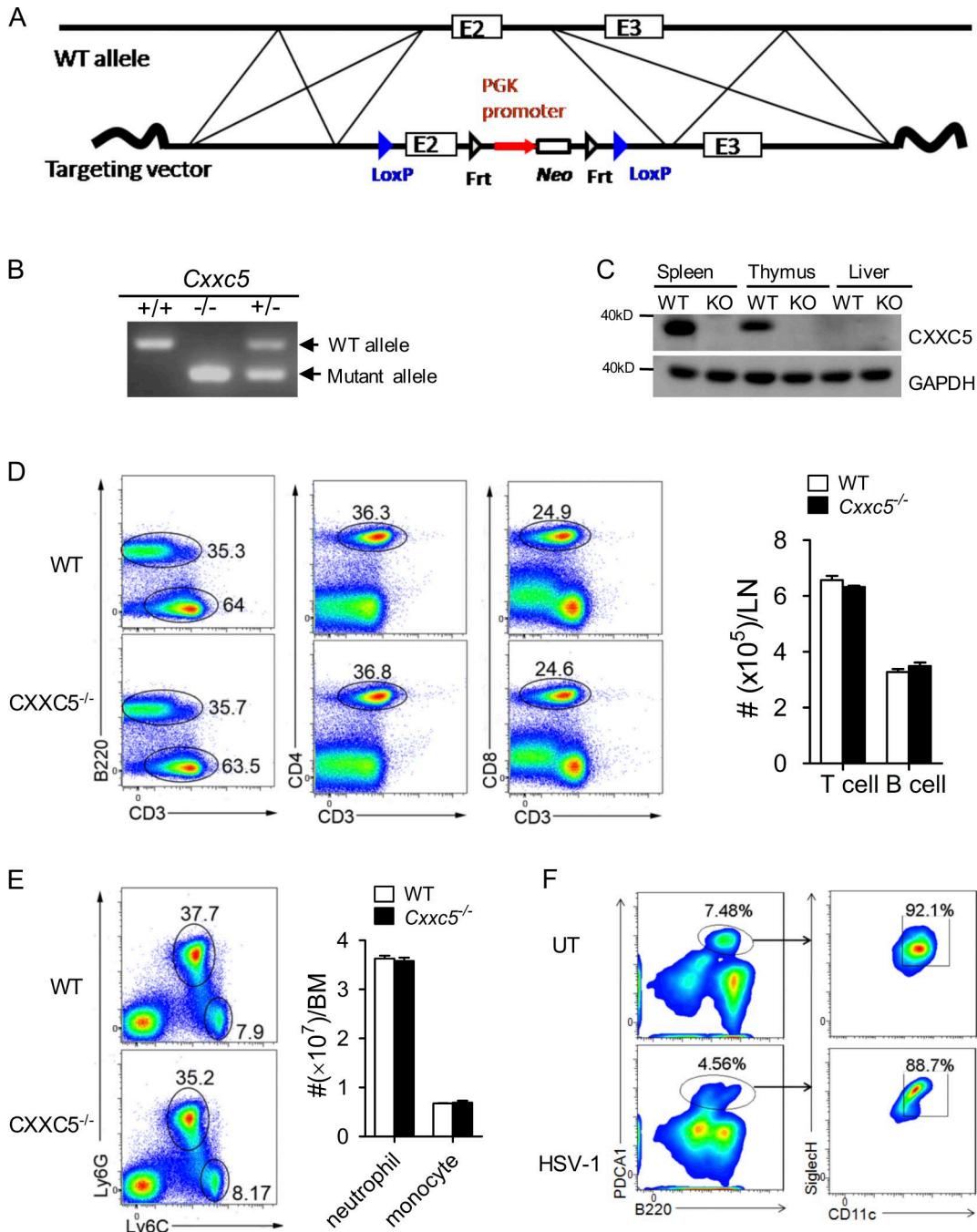
Ma et al., <https://doi.org/10.1084/jem.20161149>

Figure S1. Generation and analyses of CXXC5^{-/-} mice. (A) Graphic presentation of the gene-targeting strategy used to generate Cxxc5^{-/-} mice. ES cell line E14.1 (129/Ola-derived) was used to create Cxxc5 floxed ES cell line, which was microinjected into 129S mice. The F1 mouse carrying Cxxc5 floxed allele was crossed onto the CMV-Cre-expressing strain to generate Cxxc5-knockout mice. (B) Validation of the deletion of exon 2 from Cxxc5 gene by PCR. (C) Western blot detection of CXXC5 in various tissues isolated from WT and CXXC5^{-/-} (KO) mice. (D and E) Flow cytometry analyses of the percentages and the numbers of T and B lymphocytes from lymph nodes, as well as neutrophils and monocytes in BM. The data are presented as mean \pm SD. (F) Gating on CD11c⁺/B220⁺/PDCA1⁺/SiglecH⁺ pDCs from uninfected (UT) and HSV-infected spleen for FACS sorting.

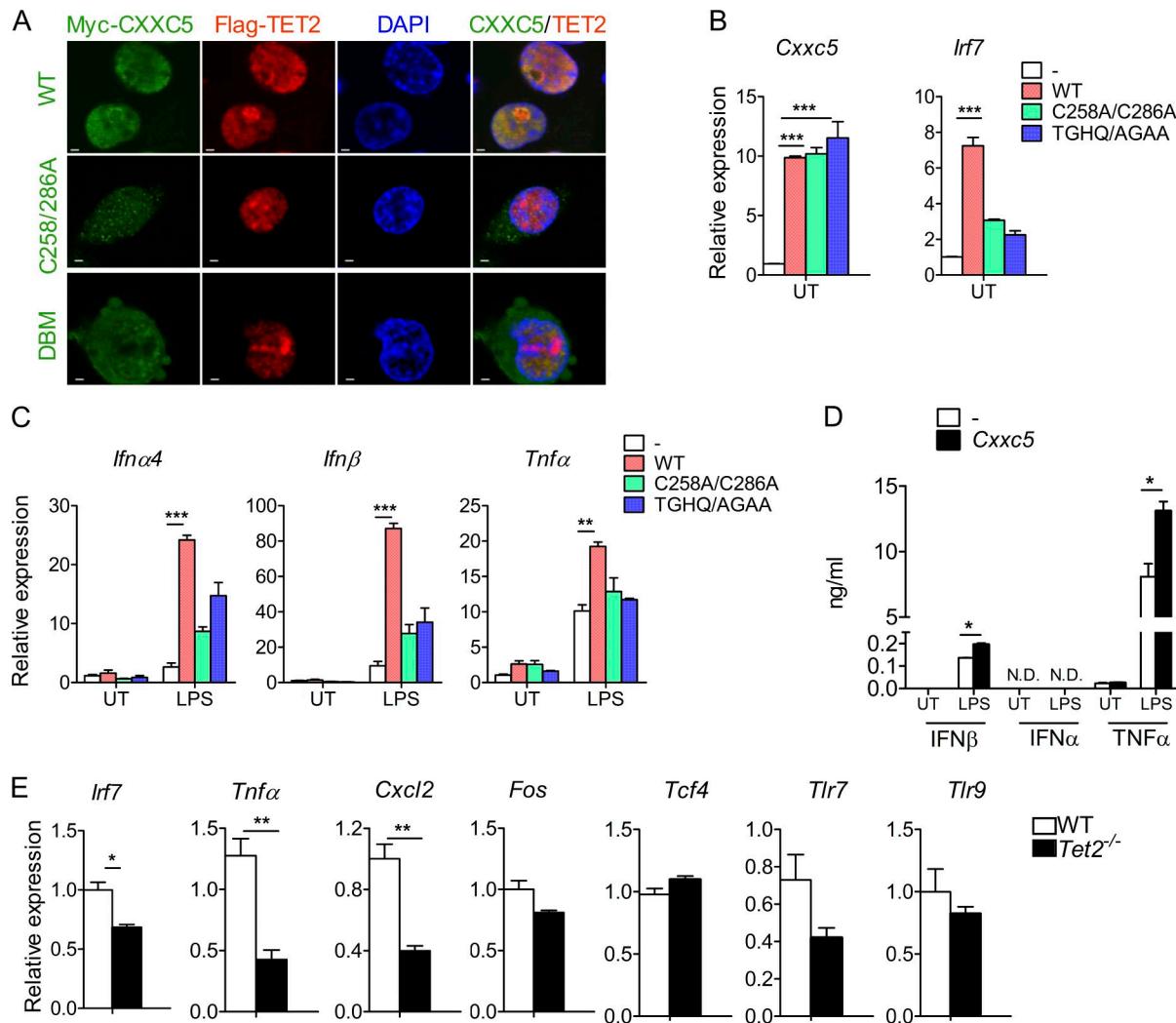


Figure S2. CXXC5-Tet2 axis promotes IFN response. (A) Plasmids expressing Flag-tagged Tet2 were cotransfected with plasmids expressing Myc-tagged WT CXXC5, Myc-tagged CXXC5-C258A/C286A mutant, or Myc-tagged CXXC5 DBM (DNA-binding deficient mutant, mutating TGHQ to AGAA) into HEK293T cells. Transfected cells were fixed in 48 h for immunofluorescence. Bars, 2 μ m. (B) CXXC5 $^{-/-}$ BMDCs were transduced by lentiviral vector pCDH-EGFP- and pCDH-EGFP-expressing WT or mutants of CXXC5. Expression levels of *Irf7* and *Cxxc5* (mean \pm SD; ***, P < 0.001) were measured by real-time PCR on sorted GFP $^{+}$ cells. UT, untreated. (C) Stable pools of BMDCs expressing WT or mutants of CXXC5 were stimulated by LPS (100 ng/ml) for 4 h, and the induction of *Ifna4*, *Ifnb*, and *Tnf α* was quantified by real-time PCR (mean \pm SD; **, P < 0.01; ***, P < 0.001). (D) Stable pools of control or CXXC5-expressing BMDCs were stimulated by LPS (100 ng/ml) for 24 h, and induction of IFN type I and TNF was measured by ELISA (mean \pm SD; *, P < 0.05). (E) Validation of subsets of genes differentially expressed in WT and *TET2* $^{-/-}$ pDCs by RT-PCR (mean \pm SD; *, P < 0.05; **, P < 0.01). All these experiments were repeated at least once with similar results.

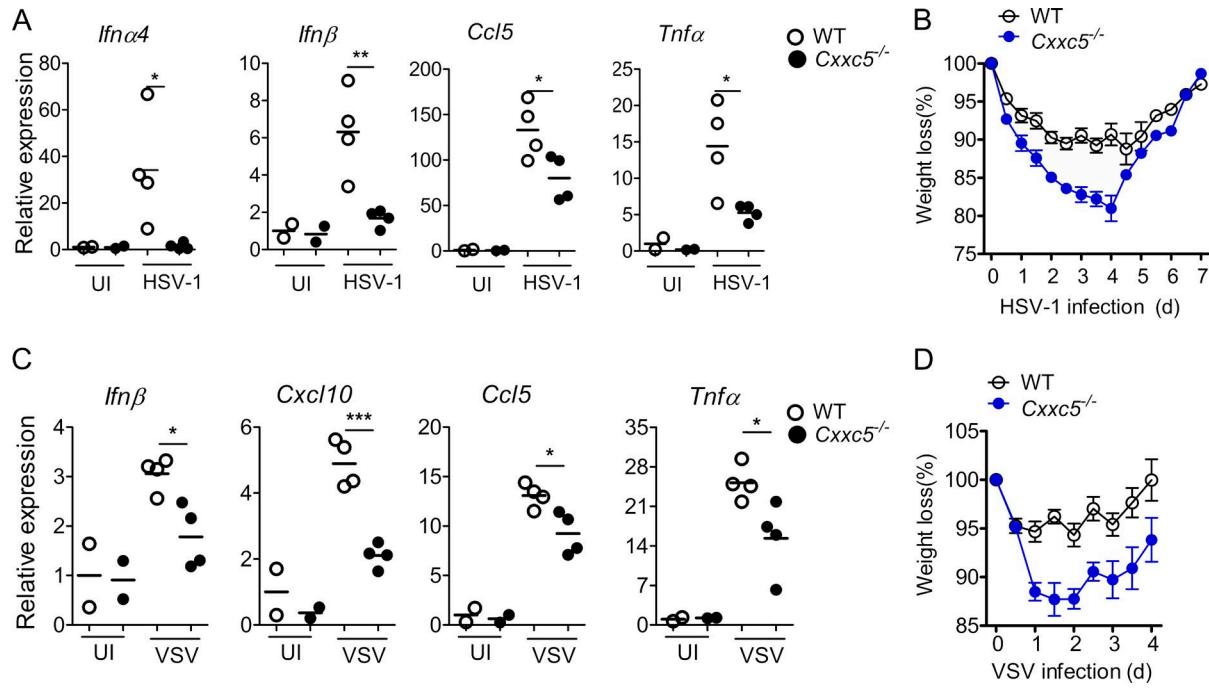


Figure S3. Viral infection-induced IFN response and morbidity in CXXC5^{-/-} mice. (A) 8-wk-old WT and CXXC5^{-/-} littermates ($n = 4$) were intravenously infected with HSV-1. Spleens were collected at 24 h after infection, and induction of *Ifnα4/β*, *Ccl5*, and *Tnfα* was measured by real-time PCR (*, $P < 0.05$; **, $P < 0.01$). (B) 6–8-wk-old WT and CXXC5^{-/-} littermates ($n = 10$) were intravenously infected with HSV-1 and monitored every 12 h for weight loss. The data (mean \pm SD) were analyzed by Student's *t* test. (C) 8-wk-old WT and CXXC5^{-/-} littermates ($n = 4$) were intravenously infected with VSV for 24 h, and splenic *Ifnβ*, *Cxcl10*, *Ccl5*, and *Tnfα* mRNAs were measured by real-time PCR (*, $P < 0.05$; ***, $P < 0.001$). (D) 6–8-wk-old WT and CXXC5^{-/-} littermates ($n = 5$) were intravenously infected with VSV and monitored every 12 h for weight loss. The data are presented as mean \pm SD and were analyzed by Student's *t* test. UI, uninfected.

Table S1. Selected gene sets regulated by CXXC5 in pDCs

Gene name	WT vs. KO (fold change)	CpGI (Y/N)	Gene name	WT vs. KO (fold change)	CpGI (Y/N)			
Steady-state pDCs								
<i>Rgs22</i>	9.47	Y	<i>Atf3</i>	2.15	Y			
<i>Ccl7</i>	8.85	N	<i>Ifih1</i>	2.14	Y			
<i>Pik3cg</i>	8.60	N	<i>Btk</i>	2.13	N			
<i>Stard5</i>	8.01	Y	<i>Il12a</i>	2.08	N			
<i>Akt1s1</i>	7.07	Y	<i>Tmem27</i>	0.17	N			
<i>Celf4</i>	6.99	Y	<i>Acan</i>	0.17	Y			
<i>Trim28</i>	6.62	Y	<i>Il3</i>	0.21	N			
<i>Tlcd1</i>	6.53	Y	<i>Map9</i>	0.21	Y			
<i>R3hdhm4</i>	6.47	Y	<i>4930546009Rik</i>	0.21	N			
<i>Patz1</i>	6.29	Y	<i>Pla2g2c</i>	0.25	N			
<i>Mfsd9</i>	6.17	Y	<i>Inhbb</i>	0.25	Y			
<i>Eri3</i>	6.11	Y	<i>Stab2</i>	0.25	N			
<i>Il2rg</i>	6.10	N	<i>Klra7</i>	0.25	N			
<i>Chrb1</i>	5.98	Y	<i>Espn</i>	0.25	N			
<i>Tnk2</i>	5.90	Y	<i>Tbc1d21</i>	0.27	N			
<i>Gm15413</i>	5.75	N	<i>Fgf8</i>	0.27	Y			
<i>Cyp27a1</i>	5.73	Y	<i>Zfp28</i>	0.27	Y			
<i>Ngp</i>	5.64	N	<i>Mcam</i>	0.27	N			
<i>Ccdc126</i>	5.63	Y	<i>2700054A10Rik</i>	0.27	N			
<i>Fam3a</i>	5.58	Y	<i>Nudt5</i>	0.37	Y			
<i>Lrif1</i>	5.57	Y	<i>Krtap5-2</i>	0.37	N			
<i>Bin2</i>	5.53	Y	<i>Olf1217</i>	0.37	N			
<i>Coro1b</i>	5.49	Y	<i>Eml1</i>	0.37	N			
<i>Edem2</i>	5.48	Y	<i>Fosl2</i>	0.37	Y			
<i>Prrg4</i>	5.47	N	<i>Wnt5b</i>	0.37	N			
<i>Vat1</i>	5.44	Y	CpG-A-activated pDCs (3 h)					
<i>Nfyb</i>	5.39	Y	<i>Rgs22</i>	13.68				
<i>Pcp4l1</i>	5.39	N	<i>Zc3h8</i>	10.38				
<i>Dynlt1a</i>	5.33	N	<i>Olf1827</i>	10.38				
<i>Itgb8</i>	5.29	Y	<i>Pfkfb2</i>	6.92				
<i>Actr1b</i>	5.27	Y	<i>Rab38</i>	6.66				
<i>Igfbpl1</i>	5.27	Y	<i>Ccl7</i>	6.63				
<i>Ptp4a3</i>	5.24	N	<i>Zfand4</i>	5.99				
<i>Armc9</i>	5.24	Y	<i>Pou1f1</i>	5.71				
<i>Ptgs2</i>	5.23	Y	<i>Edn3</i>	5.52				
<i>Slc1a5</i>	5.22	Y	<i>Klk15</i>	5.17				
<i>Tnf</i>	4.13	Y	<i>Sp8</i>	5.03				
<i>Cxcl3</i>	3.80	N	<i>Ly6g6d</i>	5.01				
<i>Pim2</i>	3.46	N	<i>Mipol1</i>	4.91				
<i>Ifitm3</i>	3.38	N	<i>Rpl31</i>	4.88				
<i>Cxcl2</i>	3.16	Y	<i>Megf9</i>	4.87				
<i>Ccl25</i>	3.00	Y	<i>Ppp1r13l</i>	4.81				
<i>Hdac1</i>	2.98	Y	<i>Tbx20</i>	4.70				
<i>Il1a</i>	2.80	N	<i>Btnl9</i>	4.65				
<i>Kdm2b</i>	2.76	Y	<i>Cmtm1</i>	4.58				
<i>Nfkb1</i>	2.71	N	<i>Folr2</i>	4.48				
<i>Ifitm2</i>	2.64	Y	<i>Prps11l</i>	4.26				
<i>Marcks1</i>	2.62	Y	<i>Eral1</i>	4.14				
<i>Nfkbb</i>	2.61	Y	<i>Slc9a5</i>	4.13				
<i>Atf6</i>	2.57	N	<i>Vps37a</i>	4.10				
<i>Ccl12</i>	2.55	N	<i>Exoc4</i>	4.08				
<i>Fos</i>	2.45	Y	<i>Grid1</i>	3.90				
<i>Cbl</i>	2.37	Y	<i>Ndst1</i>	3.82				
<i>Apoe</i>	2.36	Y	<i>Kbtbd11</i>	3.72				
<i>Jade1</i>	2.35	Y	<i>Nkx1-1</i>	3.69				
<i>Trem14</i>	2.34	N	<i>Adam23</i>	3.64				
<i>Nedd9</i>	2.32	N	<i>Ankrd13b</i>	3.60				
<i>Ccl19</i>	2.30	N	<i>Mfap3</i>	3.58				
<i>Foxp1</i>	2.30	Y	<i>Picd4</i>	3.57				
<i>Hdac10</i>	2.29	N	<i>Rgs9</i>	3.50				
<i>Irif7</i>	2.21	Y	<i>Ltbp4</i>	3.47				
<i>Syk</i>	2.19	Y	<i>Eva1c</i>	3.45				
			<i>Scgb1b19</i>	3.40				

Table S1. Selected gene sets regulated by CXXC5 in pDCs (Continued)

Gene name	WT vs. KO (fold change)	CpGI (Y/N)
<i>Slc18a2</i>	3.39	
<i>Olf1302</i>	3.35	
<i>Lrrc40</i>	3.34	
<i>Chn1</i>	3.33	
<i>Ifna12</i>	3.30	
<i>Ifna4</i>	3.24	
<i>Ifnb1</i>	2.99	
<i>Cxcl3</i>	2.38	
<i>Ccl2</i>	2.24	
<i>Wnt7a</i>	2.23	
<i>Ifna13</i>	2.18	
<i>Ifna7</i>	2.18	
<i>Siglech</i>	2.17	
<i>Pten</i>	2.16	
<i>Ifnab</i>	2.15	
<i>Ifna5</i>	2.13	
<i>Ifna14</i>	2.10	
<i>Tmem171</i>	2.09	
<i>Elf5</i>	2.09	
<i>Ifna2</i>	2.07	
<i>Ifna1</i>	2.04	
<i>Apoe</i>	1.97	
<i>Ifna11</i>	1.95	
<i>Atf7</i>	1.89	
<i>Ifitm3</i>	1.84	
<i>Ptpn1</i>	1.82	
<i>Dock2</i>	1.80	
<i>Ptpn7</i>	1.80	
<i>Slc3a2</i>	1.80	
<i>Sirt2</i>	1.77	
<i>Sox5</i>	1.76	
<i>Nfkbia</i>	1.76	
<i>Vav1</i>	1.76	
<i>Lrrc4b</i>	1.75	
<i>Ifnl3</i>	1.74	
<i>Il24</i>	1.71	
<i>Tnf</i>	1.61	
<i>Il10</i>	1.59	
<i>Atf4</i>	1.59	
<i>Hdac1</i>	1.57	
<i>Cxcl2</i>	1.52	
<i>D030004A10Rik</i>	0.14	
<i>9530059014Rik</i>	0.14	
<i>Zbed3</i>	0.14	
<i>1600027J07Rik</i>	0.15	
<i>Kcnc1</i>	0.15	
<i>Scube3</i>	0.15	
<i>A330069K06Rik</i>	0.18	
<i>Micu3</i>	0.18	
<i>Zfp7</i>	0.18	
<i>Efna4</i>	0.19	
<i>Hykk</i>	0.24	
<i>Asb8</i>	0.24	
<i>Fam160a2</i>	0.24	
<i>Gm1043</i>	0.24	
<i>Dcaf17</i>	0.24	
<i>Mdk</i>	0.30	
<i>Ms4a1</i>	0.38	
<i>Zfp811</i>	0.38	
<i>Ttll9</i>	0.38	
<i>5430427M07Rik</i>	0.38	
<i>Kap</i>	0.38	

Table S2. Primers used in this study

Gene name	Forward (5' to 3')	Reverse (5' to 3')
Primers for RT-PCR		
<i>mIfnβ</i>	GCACTGGGTGGAATGAGACTATTG	TTCTGAGGCATCAACTGACAGGT
<i>mIfnα4</i>	CTTCCCTCATGATCCTGGTAATGAT	AATCCAAAATCCTTCCTGTCTTC
<i>mCxcl10</i>	CTCATCCTGCTGGGCTGAGT	CCTATGGCCCTCATTCCTACTG
<i>mCcl5</i>	GTCCCCACGTCAGGGAGTATT	CTTCTCTGGTTGGCACACACT
<i>mTnfα</i>	GTCCTTAAAGGGATGAGAAGTT	GTTCGTCAGCTACAGTGGCTACA
<i>mIrf7-A</i>	CCAAGGAGAAGACCCGTAT	GACAAGCACAAAGCCGAGACT
<i>mIrf7-B</i>	TCTTGCCCAAGACAATT	AGCATTGCTGAGGCTCACTT
<i>mCxxc5</i>	CGAAAGACTGGCCATCAGATTT	GCCGTCACTGAATCACTGAAAC
<i>mTlr7</i>	TATCCTCTGACGCCACAAT	AAACCATCGAAACCCAAAGA
<i>mTlr9</i>	CCATCTCCAAACATGGTCT	GGCTTCAGCTCACAGGTAG
<i>mTcf4</i>	CGAATCACATGGACAGATG	ACGGGGTTAACGGAGCAGTGT
<i>mIl12b</i>	AGACCTGCCCCATTGAAGT	GAAGCTGGTGTGTAGTTCTCATATT
<i>mCxl2</i>	CGGTCAAAAAGTTGCCTTGAC	GCTCCCTTCCAGGTCACTT
<i>mIrf1</i>	CTCACCGAGAACAGAGGAAAG	GCTGCTGAGTCCATCAGAGAAA
<i>mFos</i>	ATCCGAAGGGAACGGAATAA	TGCAACGCAGACTTCTCATC
<i>mGapdh</i>	TGGAGAACCTGCCAACATG	CTGTTGAAGTCGAGGAGACAA
<i>mIFNαR1</i>	CCCCCGAGTATTGATGAGTT	GGCGCGTGTCTTACTTCTAC
<i>mIFNαR2a</i>	CGTAATGCTGAAACGGATTG	CGATGGCTTCTGAAGGTGAC
<i>mIrf9</i>	ATCTGCTGCCAGCAATAAG	TGTAGGGTTCTGGAACTTG
<i>mStat1</i>	CAGAACCGATGGAGCTTGA	CAGAACCGATGGAGCTTGA
<i>mStat2</i>	ACCAAGGAGGCAGACAAAGA	GTGCCTTGGTTTGCTGAAT
<i>hCxxc5</i>	GCTGCTTGAGAACGGTGTAG	GAGACCACACGAGCAGTGCAC
<i>hGapdh</i>	ACCCAGGTGCTCCCTCTGACT	CCCTGTTGCTGTAGCCAAATT
<i>hIrf7</i>	GGAGGCCAAGGAGAAGAG	TGCTGCTATCAGGGAAAGAC
<i>hIfnα</i>	TCATTTCTCTGCCCTGAAGG	GAGGACAGAGATGGCTTAG
<i>hIfnβ</i>	TTGACATCCCTGAGGAGATTAAGC	TTAGCCAGGAGGTTCTAACAAATAG
<i>hTet2</i>	AAAGATGAAGGTCTTTTATACC	TTTACCTCTGTCCAACACCTT
Primers for shRNA		
<i>Sh-hCxxc5</i>	CCGGGAAAGACTGGCCATCAGATTCTCGAGAAATCTGATGGCA GTCTTTCTTTTG	AATTCAAAAAGAAAGACTGGCATCAGATTCTCGAGAAATCTGATGGCA GTCTTT
<i>Sh-hTet2</i>	CCGGGCCAAGTCATTATTGACCATCTCGAGATGGTCAAATAATG ACTTGGCTTTTG	AATTCAAAAAGCCAAGTCATTATTGACCATCTCGAGATGGTCAAATAATG ACTTGGC
Primers for ChIP-qPCR/hMeDIP		
<i>Irf7-1</i>	CCACCATGCCAGTTACTT	GCTCACAAACCATCCGTAACA
<i>Irf7-2</i>	CAGGGTCAGCCCTCTCTATT	GCAAATCCTCCCTCTGT
<i>Irf7-3</i>	CCACCATGCCAGTTACTT	ACCCACAAGGTGGCTCACAAAC
<i>Irf7-4</i>	GCAGTTCTGTTTACTCGTCTG	ATTAGGGTGTGAGGGTCACAG
<i>Irf7-5</i>	GGGTCTGTGTCCTTACATTA	CACCTTGAAGATCTGTCATCT
<i>Irf7-6</i>	TCAGCCAGCTAACAAAACAAA	TTCCAGGCTTTGTAGATTCA
<i>Irf7-7</i>	GAGCCTTCTGAGGAAAAGAA	GGAGTTCAAGGTCACTTTCAT
<i>Ifnα-1</i>	CCTCCCTGTAATCCCAGTTG	GGTTGCAGAGGAAGCTTGAC
<i>Ifnα-2</i>	GAACACCTGGAGTGGAGAC	GTCCAGGGATGCAGGAAC
<i>Ifnα-3</i>	TCCCAGACACAAGCAGAGAG	TTCTCTGAGGCTCTGTG
<i>Ifnα-4</i>	CTGAGGGCAGCAGTGAAC	CCCCTGCTTCAATTCTC
<i>Actin</i>	CTCAATGCCCTCTGGTCTT	TCGATGCCAGTGTAGAGA
<i>Cxcl2</i>	GGCTCTGTGCTTCTGTATG	GAAGCTTGTGGAGGACTG
<i>Il6</i>	CCCCACCTCCAACAAAGAT	GGGCTCCAGGAGCAGAATGAG
<i>Ccl5</i>	CTTTTGTGAAACTCCCCAAGTC	GGAGATGCATGTGCTGTCTCAG
<i>Irf7</i> (bisulfite)	GTGTTATTTGGTGTATTGGTTT	AATCTATACATCTTCTCATCAAATC
<i>Cxcl2</i> (hMeDIP)	GGGCTCTGTGCTTCTGTAT	TCCCGAGAGCTCTTTATG
<i>Tnfα</i> (hMeDIP)	GATTCTTGATGCCCTGGGTGTC	GAGCTCTGTGGCTGGCTGT