

SUPPLEMENTAL MATERIAL

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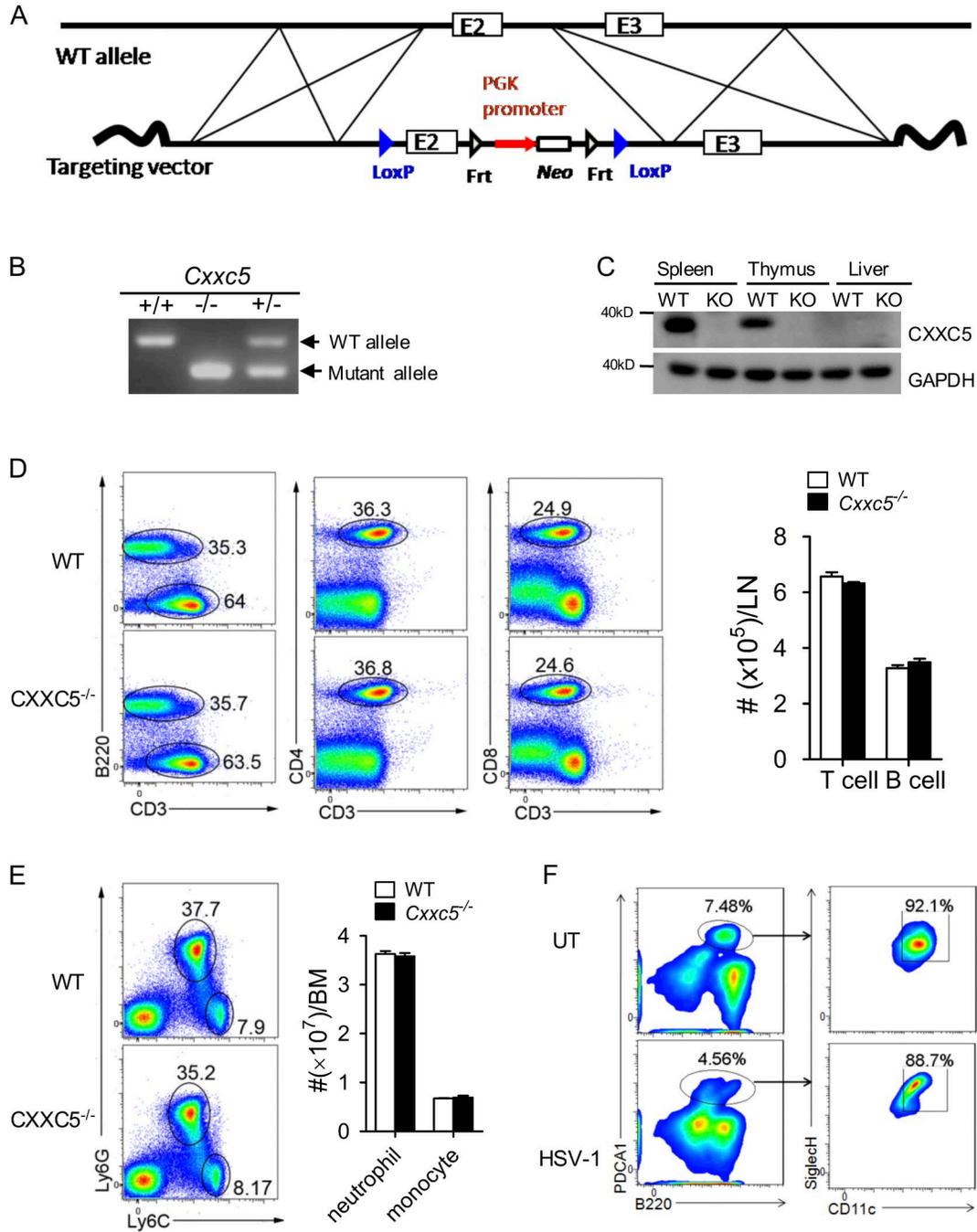


Figure S1. **Generation and analyses of CXXC5<sup>-/-</sup> mice.** (A) Graphic presentation of the gene-targeting strategy used to generate *Cxxc5*<sup>-/-</sup> mice. ES cell line E14.1 (129/Ola-derived) was used to create *Cxxc5* floxed ES cell line, which was microinjected into 129S mice. The F1 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<sup>-/-</sup> (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 ± SD. (F) Gating on CD11c<sup>+</sup>/B220<sup>+</sup>/PDCA1<sup>+</sup>/SiglecH<sup>+</sup> pDCs from uninfected (UT) and HSV-infected spleen for FACS sorting.

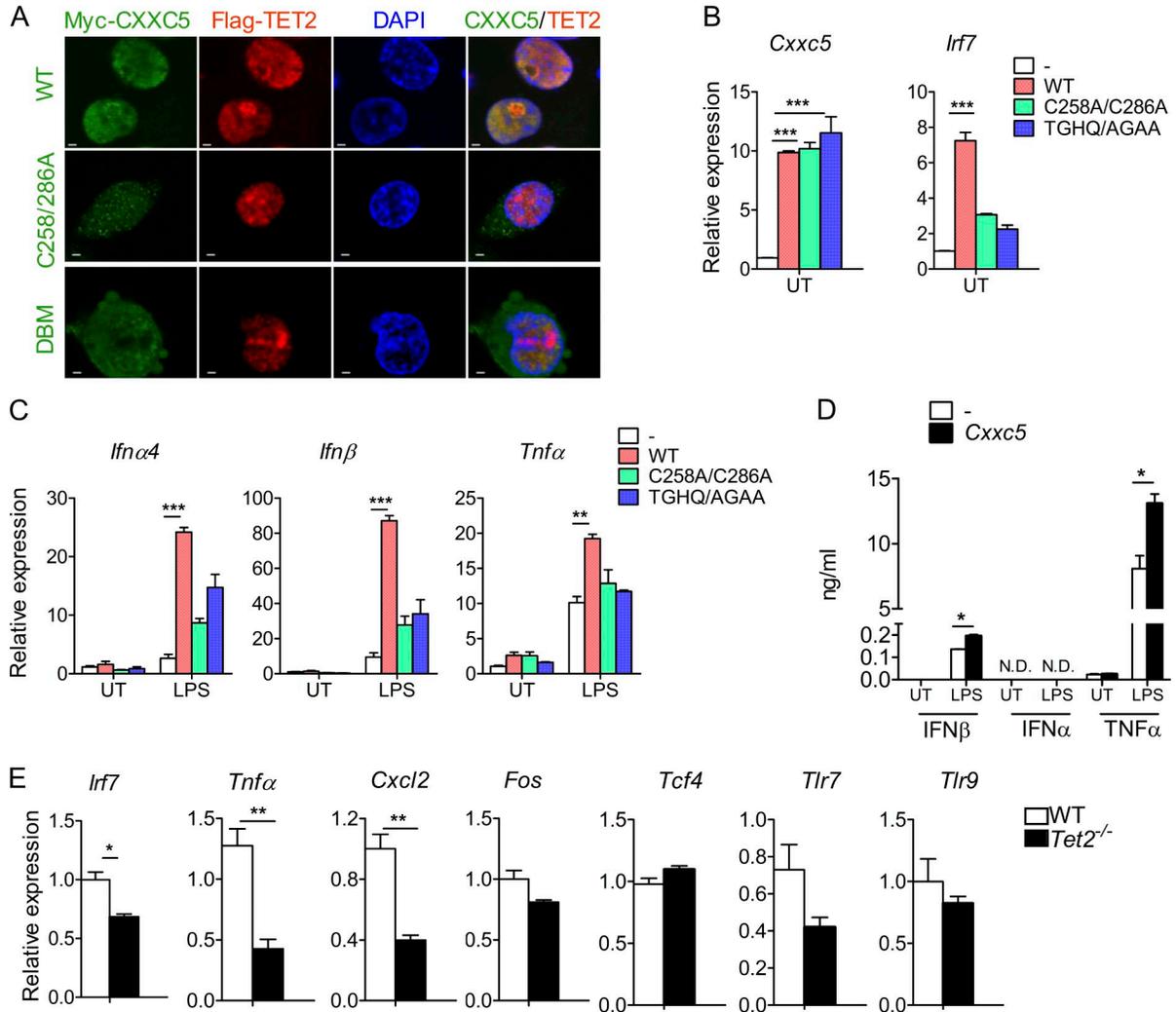


Figure S2. **CXXC5-Tet2 axis promotes IFN response.** (A) Plasmids expressing Flag-tagged Tet2 were cotransfected with plasmids expressing Myc-tagged WT CXXC5, Myc-tagged CXXC5-C258A/C286A mutant, or Myc-tagged CXXC5 DBM (DNA-binding deficient mutant, mutating TGHQ to AGAA) into HEK293T cells. Transfected cells were fixed in 48 h for immunofluorescence. Bars, 2  $\mu$ m. (B) CXXC5<sup>-/-</sup> BMDCs were transduced by lentiviral vector pCDH-EGFP<sup>-</sup> 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<sup>+</sup> 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 *Tnfa* 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<sup>-/-</sup> pDCs by RT-PCR (mean  $\pm$  SD; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ). All these experiments were repeated at least once with similar results.

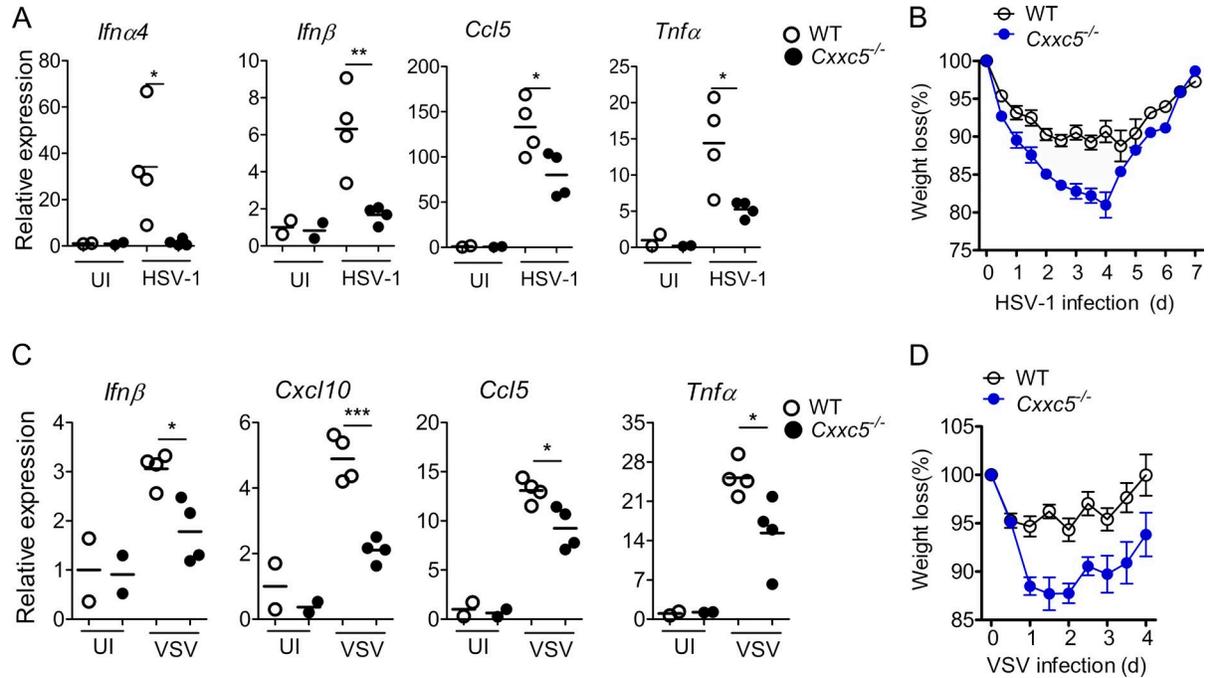


Figure S3. **Viral infection-induced IFN response and morbidity in CXXC5<sup>-/-</sup> mice.** (A) 8-wk-old WT and CXXC5<sup>-/-</sup> littermates ( $n = 4$ ) were intravenously infected with HSV-1. Spleens were collected at 24 h after infection, and induction of *Ifnα4/β*, *Ccl5*, and *Tnfα* was measured by real-time PCR (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ). (B) 6–8-wk-old WT and CXXC5<sup>-/-</sup> 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<sup>-/-</sup> 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<sup>-/-</sup> 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)
<b>Steady-state pDCs</b>			<i>Atf3</i>	2.15	Y
<i>Rgs22</i>	9.47	Y	<i>Ifih1</i>	2.14	Y
<i>Ccl7</i>	8.85	N	<i>Btk</i>	2.13	N
<i>Pik3cg</i>	8.60	N	<i>Il12a</i>	2.08	N
<i>Stard5</i>	8.01	Y	<i>Tmem27</i>	0.17	N
<i>Akt1s1</i>	7.07	Y	<i>Acan</i>	0.17	Y
<i>Celf4</i>	6.99	Y	<i>Il3</i>	0.21	N
<i>Trim28</i>	6.62	Y	<i>Map9</i>	0.21	Y
<i>Tlcd1</i>	6.53	Y	<i>4930546009Rik</i>	0.21	N
<i>R3hdm4</i>	6.47	Y	<i>Pla2g2c</i>	0.25	N
<i>Patz1</i>	6.29	Y	<i>Inhbb</i>	0.25	Y
<i>Mfsd9</i>	6.17	Y	<i>Stab2</i>	0.25	N
<i>Eri3</i>	6.11	Y	<i>Klra7</i>	0.25	N
<i>Il2rg</i>	6.10	N	<i>Espn</i>	0.25	N
<i>Chrb1</i>	5.98	Y	<i>Tbc1d21</i>	0.27	N
<i>Tnk2</i>	5.90	Y	<i>Fgf8</i>	0.27	Y
<i>Gm15413</i>	5.75	N	<i>Zfp28</i>	0.27	Y
<i>Cyp27a1</i>	5.73	Y	<i>Mcam</i>	0.27	N
<i>Ngp</i>	5.64	N	<i>2700054A10Rik</i>	0.27	N
<i>Ccdc126</i>	5.63	Y	<i>Nudt5</i>	0.37	Y
<i>Fam3a</i>	5.58	Y	<i>Krtap5-2</i>	0.37	N
<i>Lrif1</i>	5.57	Y	<i>Olfir1217</i>	0.37	N
<i>Bin2</i>	5.53	Y	<i>Eml1</i>	0.37	N
<i>Coro1b</i>	5.49	Y	<i>Fosl2</i>	0.37	Y
<i>Edem2</i>	5.48	Y	<i>Wnt5b</i>	0.37	N
<i>Prrg4</i>	5.47	N	<b>CpG-A-activated pDCs (3 h)</b>		
<i>Vat1</i>	5.44	Y	<i>Rgs22</i>	13.68	
<i>Nfya</i>	5.39	Y	<i>Zc3h8</i>	10.38	
<i>Pcp4l1</i>	5.39	N	<i>Olfir827</i>	10.38	
<i>Dynlt1a</i>	5.33	N	<i>Prkfb2</i>	6.92	
<i>Itgb8</i>	5.29	Y	<i>Rab38</i>	6.66	
<i>Actr1b</i>	5.27	Y	<i>Ccl7</i>	6.63	
<i>Igfbpl1</i>	5.27	Y	<i>Zfand4</i>	5.99	
<i>Ptp4a3</i>	5.24	N	<i>Pou1f1</i>	5.71	
<i>Armc9</i>	5.24	Y	<i>Edn3</i>	5.52	
<i>Ptgs2</i>	5.23	Y	<i>Klk15</i>	5.17	
<i>Slc1a5</i>	5.22	Y	<i>Sp8</i>	5.03	
<i>Tnf</i>	4.13	Y	<i>Ly6g6d</i>	5.01	
<i>Cxcl3</i>	3.80	N	<i>Mipol1</i>	4.91	
<i>Pim2</i>	3.46	N	<i>Rpl3l</i>	4.88	
<i>Ifitm3</i>	3.38	N	<i>Megf9</i>	4.87	
<i>Cxcl2</i>	3.16	Y	<i>Ppp1r13l</i>	4.81	
<i>Ccl25</i>	3.00	Y	<i>Tbx20</i>	4.70	
<i>Hdac1</i>	2.98	Y	<i>Btnl9</i>	4.65	
<i>Il1a</i>	2.80	N	<i>Cmtm1</i>	4.58	
<i>Kdm2b</i>	2.76	Y	<i>Folr2</i>	4.48	
<i>Nfkb1</i>	2.71	N	<i>Prps11l</i>	4.26	
<i>Ifitm2</i>	2.64	Y	<i>Eral1</i>	4.14	
<i>Marcks1l</i>	2.62	Y	<i>Slc9a5</i>	4.13	
<i>Nfkbib</i>	2.61	Y	<i>Vps37a</i>	4.10	
<i>Atf6</i>	2.57	N	<i>Exoc4</i>	4.08	
<i>Ccl12</i>	2.55	N	<i>Grid1</i>	3.90	
<i>Fos</i>	2.45	Y	<i>Ndst1</i>	3.82	
<i>Cbl</i>	2.37	Y	<i>Kbtbd11</i>	3.72	
<i>Apoe</i>	2.36	Y	<i>Nkx1-1</i>	3.69	
<i>Jade1</i>	2.35	Y	<i>Adam23</i>	3.64	
<i>Trem14</i>	2.34	N	<i>Ankrd13b</i>	3.60	
<i>Nedd9</i>	2.32	N	<i>Mfap3</i>	3.58	
<i>Ccl19</i>	2.30	N	<i>Plcd4</i>	3.57	
<i>Foxp1</i>	2.30	Y	<i>Rgs9</i>	3.50	
<i>Hdac10</i>	2.29	N	<i>Ltbp4</i>	3.47	
<i>Irf7</i>	2.21	Y	<i>Eva1c</i>	3.45	
<i>Syk</i>	2.19	Y	<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>Olfrl302</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>Nfkb1</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>Kenc1</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')
<b>Primers for RT-PCR</b>		
<i>mlfn<math>\beta</math></i>	GCACTGGGTGGAATGAGACTATTG	TTCTGAGGCATCAACTGACAGGTC
<i>mlfn<math>\alpha</math>4</i>	CTTTCCTCATGATCCTGGTAATGAT	AATCCAAAATCCTTCTGTCCTTC
<i>mCxcl10</i>	CTCATCTGCTGGGTCTGAGT	CCTATGGCCCTCATTCTCACTG
<i>mCcl5</i>	GTGCCACGTC AAGGAGTATTT	CTTCTCTGGTTGGCACACACT
<i>mTnfa</i>	GTCCCCAAAGGGATGAGAAGTT	GTTTGCTACGACGTGGGTACA
<i>mlrf7-A</i>	CCCCAAGAGAAGACCCTGAT	GACAAGCACAGCCGAGACT
<i>mlrf7-B</i>	TCTTGCGCCAAGACAATTC	AGCATTGCTGAGGCTCACTT
<i>mCxxc5</i>	CGAAAGACTGGCCATCAGATTT	GCCGCTCACTGAAATCACTGAAAC
<i>mTlr7</i>	TATCCTCTGACCGCCACAAT	AAACCATCGAAACCCAAAGA
<i>mTlr9</i>	CCATCTCCCAACATGGTTCT	GGCTTCAGCTCACAGGGTAG
<i>mTcf4</i>	CGAATCATATGGGACAGATG	ACGGGGTTAAGGAGCAGTGT
<i>ml112b</i>	AGACCCTGCCATTGAACTG	GAAGCTGGTGCTGTAGTTCTCATATT
<i>mCxcl2</i>	CGGTCAAAAAGTTTGCCTTGAC	GCTCCTCCTTCCAGGTCAGTT
<i>mlrf1</i>	CTCACAGGAACAGAGGAAAAG	GCTGCTGAGTCCATCAGAGAAA
<i>mFos</i>	ATCCGAAGGGAACGGAATAA	TGCAACGCAGACTTCTCATC
<i>mGapdh</i>	TGGAGAAACCTGCCAAGTATGA	CTGTTGAAGTCGCAGGAGACAA
<i>mlFN<math>\alpha</math>R1</i>	CCCCGCAGTATTGATGAGTT	GGCGCGTGCTTACTTCTAC
<i>mlFN<math>\alpha</math>R2a</i>	CGTAATGCTGAAACGGATTG	CGATGGCTTCTGAAGGTGAC
<i>mlrf9</i>	ATCTGCTGCCAGCAATAAG	TGTAGGGTTGCTTGGAACTTG
<i>mStat1</i>	CAGAACCAGTGGAGCTTGA	CAGAACCAGTGGAGCTTGA
<i>mStat2</i>	ACCAAGGAGGCAGACAAAAGA	GTGCCTTGGTTTTGCTGAAT
<i>hCxxc5</i>	GCTGCTCTGGAGAAGGTGATG	GAGACCACAGCAGTGCACA
<i>hGapdh</i>	CACCAGTGGTCTCCTCTGACT	CCCTGTGCTGTAGCCAAATTC
<i>hlrf7</i>	GGAGGCCAAGGAGAAGAG	TGCTGCTATCCAGGGAAGAC
<i>hlfn<math>\alpha</math></i>	TCATTTCTCCTGCCTGAAGG	GAGGACAGAGATGGCTTGA
<i>hlfn<math>\beta</math></i>	TTGACATCCCTGAGGAGATTAAGC	TTAGCCAGGAGTTCTCAACAATAG
<i>hTet2</i>	AAAGATGAAGTCTTTTTATACCC	TTTACCCTTCTGTCCAAACCTT
<b>Primers for shRNA</b>		
<i>Sh-hCxxc5</i>	CCGGAAAGACTGGCCATCAGATTTCTCGAGAAATCTGATGGCCA GTCCTTCTTTTTG	AATTCAAAAGAAAGACTGGCCATCAGATTTCTCGAGAAATCTGATGGCCA GTCCTTTC
<i>Sh-hTet2</i>	CCGGGCCAAGTCATTATTTGACCATCTCGAGATGGTCAAATAATG ACTTGGCTTTTTG	AATTCAAAAGCCAAGTCATTATTTGACCATCTCGAGATGGTCAAATAATG ACTTGGC
<b>Primers for ChIP-qPCR/hMeDIP</b>		
<i>lrf7-1</i>	CCACCATGCCAGTTTACTT	GCTCACACCATCCGTAACA
<i>lrf7-2</i>	CAGGGTCAGCCTCTCTCATT	GCAAATCCTTCCCTCCTGTT
<i>lrf7-3</i>	CCACCATGCCAGTTTACTT	ACCACAAGGTGGCTCACAAAC
<i>lrf7-4</i>	GCAGTTTCGTTTTACTCGTCTG	ATTAGGGTGTGAGGGTCACAG
<i>lrf7-5</i>	GGGTCTGTGTCCTTACATTA	CACCTTGAAGATCTGTGCATCT
<i>lrf7-6</i>	TCAGCCAGCTAAACAAAACAAA	TTCCAGGCTCTTTGTAGATTCA
<i>lrf7-7</i>	GAGCCTTTCTGAGGAAAAGAA	GGAGTTTCAAGGTCACTTTTCAT
<i>lfn<math>\alpha</math>-1</i>	CCTCCCTGTAATCCCAGTTG	GGTTGCAGAGGAAGCTTGAC
<i>lfn<math>\alpha</math>-2</i>	GAACACCTGGGAGTGGAGAC	GTCCAGGGATGCAGGAAAC
<i>lfn<math>\alpha</math>-3</i>	TCCCAGACACAAGCAGAGAG	TTCTCTGAGAGCCTGCTGTG
<i>lfn<math>\alpha</math>-4</i>	CTGAGGGCAGCAGTAAACT	CCCCTTGCTTTCCAATTCTC
<i>Actin</i>	CTCAATGGCCTCTGGGTCTT	TCGATGCCAGTGATAGAGA
<i>Cxcl2</i>	GGCTCTGTGCTTCTCTGATG	GAAGCTTGTGGAGGCACTG
<i>Il6</i>	CCCCACCCTCCAACAAAGAT	GGGCTCCAGAGCAGAATGAG
<i>Ccl5</i>	CTTTTGTGAAACTCCCCAAGTC	GGAGATGCATGTGCTGTCTCAG
<i>lrf7 (bisulfite)</i>	GTTTATTTTGGTTGATTGGGTTTT	AATCTATACATCTTCTCATCCAAATC
<i>Cxcl2 (hMeDIP)</i>	GGGCTCTGTGCTTCTCTGAT	TCCCAGAGCTCCTTTTATG
<i>Tnfa (hMeDIP)</i>	GATTCCTTGATGCCTGGGTGTC	GAGCTTCTGCTGGCTGGCTGT