









Η



Figure S2





Figure S3







С









Figure 5



С

Figure 6

Α

в

JASPAR database predict binding sites on CCL3 promoter

Matrix ID	Name	Score	Relative score Se	quence ID	Start	End	Strand	Predicted sequence
MA0137.3	STAT1	17.1267	0.994432864867	seq1	359	369	-	TTTCTAGGAAA
MA0137.3	STAT1	10.535	0.904365664294	seq1	359	369	+	TTTCCTAGAAA
MA0137.3	STAT1	9.75502	0.893708366085	seq1	1024	1034	+	TTTCTGGCAAA
MA0137.3	STAT1	7.03215	0.856503982496	seq1	701	711	+	CTTATGAGAAG
MA0137.3	STAT1	6.67335	0.851601411616	seq1	399	409	+	TTTCTAGGCAC
MA0137.3	STAT1	6.42874	0.848259105628	seq1	1023	1033	-	TTGCCAGAAAA
MA0137.3	STAT1	5.60335	0.836981317486	seq1	997	1007	-	ATACTGGGAAG
MA0137.3	STAT1	4.73249	0.825082068621	seq1	967	977	-	TTTATAGGCAG
MA0137.3	STAT1	4.57772	0.822967464205	seq1	399	409	-	GTGCCTAGAAA
MA0137.3	STAT1	4.29079	0.819046900821	seq1	841	851	+	ATTCTTGGATA
MA0137.3	STAT1	3.96977	0.814660505768	seq1	841	851	-	TATCCAAGAAT
MA0137.3	STAT1	2.90439	0.800103467253	seq1	245	255	-	CATCAGGGAAT
Matrix ID	Name	Score	Relative score Seq	uence ID	Start	End	Strand	Predicted sequence
MA0144.2	STAT3	14.8588	0.977562698655	seq1	359	369	-	TTTCTAGGAAA
MA0144.2	STAT3	11.2844	0.934260425347	seq1	359	369	+	TTTCCTAGAAA
MA0144.2	STAT3	9.88568	0.917315339214	seq1	1023	1033	-	TTGCCAGAAAA
MA0144.2	STAT3	8.97764	0.906314781124	seq1	399	409	-	GTGCCTAGAAA
MA0144.2	STAT3	8.35767	0 80880/163200					
MA0144.2		0.00101	0.090004103299	seq1	997	1007	-	ATACTGGGAAG
	STAT3	8.33808	0.89856684616	seq1 seq1	997 701	1007 711	- +	ATACTGGGAAG CTTATGAGAAG
MA0144.2	STAT3 STAT3	8.33808 7.49031	0.89856684616 0.888296505317	seq1 seq1 seq1	997 701 1024	1007 711 1034	- + +	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA
MA0144.2 MA0144.2	STAT3 STAT3 STAT3	8.33808 7.49031 7.04255	0.89856684616 0.888296505317 0.882872085497	seq1 seq1 seq1 seq1	997 701 1024 245	1007 711 1034 255	- + + -	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT
MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205	0.89856684616 0.888296505317 0.882872085497 0.86685606691	seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360	1007 711 1034 255 370	- + - +	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT
MA0144.2 MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205 4.72779	0.89856684616 0.888296505317 0.882872085497 0.86685606691 0.854829791816	seq1 seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360 967	1007 711 1034 255 370 977	- + - +	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT CTGCCTATAAA
MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205 4.72779 4.70663	0.89856684616 0.888296505317 0.882872085497 0.86685606691 0.854829791816 0.854573434773	seq1 seq1 seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360 967 841	1007 711 1034 255 370 977 851	- + - +	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT CTGCCTATAAA TATCCAAGAAT
MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205 4.72779 4.70663 4.56273	0.89856684616 0.888296505317 0.882872085497 0.86685606691 0.854829791816 0.854573434773 0.852830168758	seq1 seq1 seq1 seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360 967 841 841	1007 711 1034 255 370 977 851 851	- + - + + -	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT CTGCCTATAAA TATCCAAGAAT ATTCTTGGATA
MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205 4.72779 4.70663 4.56273 3.68747	0.89856684616 0.888296505317 0.882872085497 0.8685606691 0.854829791816 0.854573434773 0.852830168758 0.84222674226	seq1 seq1 seq1 seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360 967 841 841 5	1007 711 1034 255 370 977 851 851 15	- + - + + -	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT CTGCCTATAAA TATCCAAGAAT ATTCTTGGATA AAGCATGGAAA
MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205 4.72779 4.70663 4.56273 3.68747 3.0285	0.89856684616 0.888296505317 0.882872085497 0.8685606691 0.854829791816 0.854573434773 0.852830168758 0.84222674226 0.834243613082	seq1 seq1 seq1 seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360 967 841 841 5 543	1007 711 1034 255 370 977 851 851 15 553	- + - + - + -	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT CTGCCTATAAA TATCCAAGAAT ATTCTTGGATA AAGCATGGAAA CTGAATGAAAG
MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2 MA0144.2	STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3 STAT3	8.33808 7.49031 7.04255 5.7205 4.72779 4.70663 4.56273 3.68747 3.0285 2.42992	0.89856684616 0.888296505317 0.868569505317 0.8685696691 0.854829791816 0.854573434773 0.852830168758 0.84222674226 0.834243613082 0.826992093329	seq1 seq1 seq1 seq1 seq1 seq1 seq1 seq1	997 701 1024 245 360 967 841 841 5 543 399	1007 711 1034 255 370 977 851 851 15 553 409	- + - + + - + -	ATACTGGGAAG CTTATGAGAAG TTTCTGGCAAA CATCAGGGAAT TTCCTAGAAAT CTGCCTATAAA TATCCAAGAAT ATTCTTGGATA AAGCATGGAAA CTGAATGAAAG TTTCTAGGCAC

CCL3 gene : 3'UTR Pro +0.5kb -1.5kb -1kb -0.5kb -0.5kb -1.0kb 1 Primer 1 -Primer 2 • Primer 3 Primer 4 Primer 5 -Primer 6 -Primer 7 -



1 Supplementary Figures Legends.

2 Figure S1. In-vitro migration of monocytes and macrophages using MCL-conditioned

medium. (A-C) Human monocytic cells, CD14+ Mo (n=3), THP1, and U937 cells were 3 incubated with conditioned medium (CM) collected from human MCL cell lines (JVM2, Mino, or 4 Granta), and migration was assessed by chemotaxis assay as described in the Methods 5 6 section. (D-F) Murine BMDM (n=3), PEM (n=3), and RAW264.7 were incubated with CM collected from FC- muMCL1 and normal mouse splenic B cells (n=2), and the migration was 7 assessed by chemotaxis assay. *p<.05, **p<.01, *p<.001 vs media alone. Experiments were 8 performed three times independently. (G) The mRNA level of CCL3 in monocytes (THP1 and 9 U937) and MCL (Mino and JVM2) was assessed by QRT-PCR. The data presented are 10 representative of three independent experiments. (H) THP1 and U937 - Mo was treated with 11 12 recombinant CCL3 (rCCL3) as indicated in the figure, and migration was assessed by chemotaxis. 13

Figure S2. CCR1 expression in MCL cells. (A-C) The expression of CCR1 or CCR5 in Mino
 and JVM2 (A) or FC-muMCL1 (B) was measured by flow cytometry. (C) The bioinformatics
 analysis of CCR1 expression in normal B cells and MCL.

Figure S3. PEM in CCR1-/- and WT mice. (A) The morphology of BMDM and PEM was collected from WT and CCR1-/- mice (n=4). (B) The expression of CD11b+ and F4/80 in WT and CCR1-/- C57/BL/6NJ mouse PEM was measured by flow cytometry. The experiment was performed in 3 mouse tumors and a representative dot plot is shown. (C) CD14+ cells were treated with 2 different CCR1 siRNA and expression of CCR1 was measured by QRT-PCR.

Figure S4. Effect of exogenous CCL3 on M1 and M2 markers in WT and CCR1-/- PEM. (A-B)_The mRNA level of CD86 was assessed in response to rCCL3 in WT and CCR1-/- PEM (A) or WT and CCR1-/- BMDM (B) by qRT-PCR.

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Figure S5. Expression of IL-10 and IL-10Rα in MCL and macrophages /monocytes. (A-B) Bioinformatics analysis of IL-10Rα and IL-10Rβ in normal B cells and various NHLs subtypes were assessed by the Oncomine database. (C) The expression of IL-10Rα in CD14-Mo and THP1- Mo was measured by flow cytometry. The experiment was repeated 2 times with similar results. (D) Mino and JVM2 were treated with indicated concentrations of rIL-10 for 48 hours, and the mRNA level of IL-10Rα and IL-10Rβ were assessed by QRT-PCR.

Figure S6. Prediction of binding sites on CCL3 promoter. (A) The predicted binding site of STAT1 and STAT3 on the CCL3 promoter was obtained from the JASPAR database. (B) Based on the CCL3 promoter sequence obtained from the UCSC genome browser, 7 pairs of primers were designed.

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Supplementary Tables.

Antibodies	Company	Catalog number
Ly-6C	Santa Cruz, USA	sc-271811
Ly-6G/Gr-1	Santa Cruz, USA	sc-53515
IL-10	Proteintech, USA	60269-1-lg
CD206	Abcam, USA	ab64693
CCR1	ABclonal, USA	A18341
IL-10Rα	Santa Cruz, USA	sc-365374
CD19	Abcam, USA	ab227019
CD8	Santa Cruz, USA	sc-18860
CD11b	Santa Cruz, USA	sc-1186
F4/80	Abcam, USA	ab111101

Table S1. Antibodies used for Immunofluorescence.

<u>Table S2.</u> Antibodies used for flow cytometry.

Antibodies	Company	Catalog number
hCCR1	R&D systems, USA	FAB145P
hCCR4	R&D systems USA	FAB1567P
hCCR5	R&D systems, USA	FAB182P
mCCR1	R&D systems, USA	FAB6280P
hIL-10Rα	R&D systems, USA	FAB5986P
mF4/80	Biolegend	123141
mCD11b	Biolegend	101206

Table S3. Primer sequences for qRT-PCR.

	Famurand	Deviewee
primers	Forward	Reverse
hIL-10	GACTTTAAGGGTTACCTGGGTTG	TCACATGCGCCTTGATGTCTG
hCCL3	AGTTCTCTGCATCACTTGCTG	CGGCTTCGCTTGGTTAGGAA
hCD206	ACCTCACAAGTATCCACACCATC	CTTTCATCACCACACAATCCTC
hCD80	CTGCCTGACCTACTGCTTTG	GGCGTACACTTTCCCTTCTC
IL-10Rα	CCTCCGTCTGTGTGGTTTGAA	CACTGCGGTAAGGTCATAGGA
IL-10Rβ	ATGAGCATTCAGACTGGGTAAAC	TTTTAGGGGCTAAGAAACGCAT
hGAPDH	ATCACCATCTTCCAGGAGCG	CAAATGAGCCCCAGCCTTC
mCD206	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
mCD80	CCCCAGAAGACCCTCCTGATAG	CCGAAGGTAAGGCTGTTGTTTG
mIL-10	AAGGCAGTGGAGCAGGTGAA	CCAGCAGACTCAATACACAC
mCCL3	ACTGCCTGCTGCTTCTCCTACA	ATGACACCTGGCTGGGAGCAAA
mCCR1	GCCAAAAGACTGCTGTAAGAGCC	GCTTTGAAGCCTCCTATGCTGC
mGAPDH	CCCACTCTTCCACCTTCGATG	GTCCACCACCCTGTTGCTGTAG

Table S4. Primer sequences for ChIP assay

CCL3 promoter primers	Forward	Reverse
Primer 1	ATGCTTAGGGTTGGCAAGGA	AGTCTCTAAAATGAATGAAGTGGCA
Primer 2	AAGCATATTTATGCCACTTCATT	CCCAAATATGGAGCCATCTTCAA
Primer 3	GGCTCCATATTTGGGTTGTTTCC	TAGTGACTAGGGCGCTGTGT
Primer 4	GCGCCCTAGTCACTACATGA	CCACAGGGATAGGGTTGATGG
Primer 5	CCCATCAACCCTATCCCTGTG	GGGAAATGGTTTCTCCTGTGAG
Primer 6	TCTTCACACTCACAGGAGAAACCA	GGGGGTGAGGAGGGAAATTTTTA
Primer 7	CTCCTCACCCCCAGATTCCATT	CCTCCATTTCACCTCTTCCTAATCT