Supplementary Data

Supplementary Figure 1. Knockdown of VentX with a different siRNA sequence reduces terminal monocyte to macrophage differentiation. Monocytes were transfected with either a scrambled sequence of siRNA (Scr siRNA) or VentX siRNA-2 (5'-UCUACUCAACGUCUUCUGGCCUUGCCAAU-3') through electroporation. Mock transfection that did not include siRNA was also conducted as control. Overnight after transfection, cells were exposed to 100ng/mL M-CSF to trigger their macrophagic differentiation. (A) At 3 days after transfection, cells were collected to analyze the efficiency of VentX knockdown by real-time PCR. (B) At 4 days after transfection, FACS analysis was performed with cells stained with PE-conjugated anti-CD71 antibody. (C) Cells were stained with PE-conjugated anti-CD14, CD64, TLR4, CD80 and CD11c.

Supplementary Figure 2. FACS analysis of monocyte phenotypes after VentX knockdown. Monocytes were transfected with either siGFP or siVentX and subsequently exposed to 100ng/mL M-CSF to trigger their macrophagic differentiation. At 4 days after transfection, cells were collected and stained with indicated antibodies followed by FACS analysis.

Supplementary Figure 3. Time-course expression of CD71 in primary monocytes after VentX knockdown. Monocytes were transfected with either siGFP or siVentX and subsequently exposed to 100ng/mL M-CSF to trigger their macrophagic differentiation. Surface expression of CD71 was determined by FACS analysis at indicated days after siRNA transfection. Upper panel shows the percentage of CD71 positive cells and lower panel shows the normalized MFI of CD71 staining.

Supplementary Figure 4. VentX is required for macrophage differentiation of primary monocytes in GM-CSF and IL3 cultures. Monocytes were transfected with either siGFP or siVentX and subsequently exposed to 100ng/mL GM-CSF (A) or IL3 (B) to trigger macrophagic differentiation. Surface expression of CD71 was determined by FACS analysis at 4 days after siRNA transfection. . Filled blue histogram represents the isotope control staining; red histogram: monocytes transfected with siGFP; green histogram: monocytes transfected with siVentX.

Supplementary Figure 5. Homeodomain (HD) of VentX is essential for inducing macrophagic differentiation in U937 cells. (A) A schematic diagram depicts various truncated VentX constructs (all in pRetroX-Tight-Puro retroviral vector) used in this study. (B) The U937 cell lines conditionally expressing three truncated VentX were generated as described in Materials and Methods. Cell surface markers were detected by FACS analysis after 3 days exposure to 1.0 μg/mL DOX.

Supplementary Figure 6. Screening for downstream targets of VentX associated with monocyte differentiation in HL60 cells. HL60 cells were transfected with plasmids encoding GFP or GFPVentX. Positively transfected cells were sorted and treated with 10nM PMA to induce monocytic differentiation. At indicated time points after PMA treatment, cells were harvested for quantitative analysis of mRNA level of PU1, Foxp1, C/EBPα, MafB, Egr-1, AML1, IRF-8 and M-CSFR. The value at zero time point was designated as 1.0.

Supplementary Figure 7. (A) Schematic diagram depicts promoter region of M-CSF receptor. (B) Schematic diagram showing different transcriptional factors binding sites on M-CSFR promoter region.

Supplementary Figure 8. Complementation of M-CSF receptor partially rescued macrophage differentiation defect in VentX siRNA transfected monocytes. Freshly isolated monocytes were transfected with the indicated combination of siRNA and plasmid through electroporation. At 4 days after transfection, cells were harvested and stained with anti-CD71 antibody followed by FACS analysis.

Supplementary Figure 9 Analysis of macrophage phenotypes after VentX knockdown. Macrophages were derived from fresh monocytes by incubation with 100ng/mL M-CSF for 4 days. Macrophages were then collected and transfected with siGFP or siVentX, respectively, and further incubated in M-CSF culture for 3 days. (A) The photographs of cells were taken directly in culture using phase-contrast microscopy. The percentage of cells with adhesion was calculated as described in Fig. 3B. The percentages of cells with a fibroblastic shape were counted as cells with morphological change. (B) Macrophages were harvested and stained with indicated antibodies followed by FACS analysis.

Supplementary Figure 10. VentX is not essential for macrophage alternative activation. Macrophages were treated as described in Supplementary Figure 11. 100ng/ml of IL4 was added to culture 24h before harvest. (A) Total RNA was isolated to analyze the mRNA level of CD163, IL10, MR and CCL-18 by real-time PCR. (B) Cells were harvested and stained by anti-MR and anti-CD163 antibodies followed by FACS analysis.

Supplementary Figure 11. p300 is required for VentX induced pro-inflammatory response in U937 cells. U937 cells were first transfected with p300 siRNA or control siRNA through electroporation. At 24 hours after transfection, cells were treated with DOX to induce GPF or GFPVentX expression. At 72 hours after transfection, cells were harvested to determine mRNA level of TNF-α and IL1-β.

Supplementary Figure 12. VentX expression is down-regulated in SLE/RA patients upon immunosuppressant treatment. SLE/RA patients receiving immunosuppressant such as Imuran,

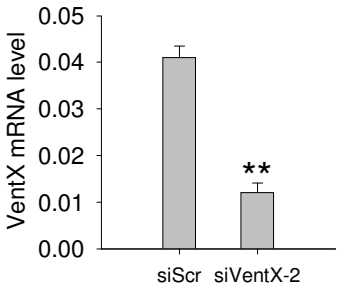
Methotrexate or Prednisone were classified as treatment group, while patients not receiving immunosuppressant were classified as control group. (A) Total RNA was isolated from peripheral blood leukocytes of SLE/RA patients. Quantitative measurements of mRNA levels of VentX and the indicated cytokines were performed as described in Materials and Methods. mRNA levels were represented in box plots indicating the median and the lower and upper quartiles. Statistically, significant differences between the immunosuppressant-receiving group and the control group were revealed by Wilcoxon rank-sum test. (B) Total RNA was purified from peripheral monocytes of SLE/RA patients and same analysis was performed as above.

Supplementary Figure 13. ECR browser analysis of VentX loci among human, chimpanzee and rhesus monkey. (A) The VentX loci of human, chimpanzee (panTro2) and rhesus monkey (rheMac2) were analyzed by ECR browser (http://ecrbrowser.dcode.org). The human genomic sequence of chr10:134898423-134905423 (~7 Kb), which encompasses the VentX locus, was used as base genome. VentX gene is depicted as a horizontal blue line above the graph, with strand/transcriptional orientation indicated by arrow. Blue boxes along the line correspond to positions of coding exons, while yellow boxes correspond to UTRs. Peaks within the conservation profile that correspond to these exons are similarly colored within the plot. Peaks within the conservation profile that do not correspond to transcribed sequences are highlighted in red if they are intergenic or salmon if they lie within an intron. Regions colored in green correspond to transposable elements and simple repeats. The sequence of the base genome is represented on the horizontal axis, and the vertical position corresponds to the level of nucleotide identity in this alignment. (B) The promoter region of VentX loci (Shown in Red) was analyzed for conserved transcriptional factors binding sites from TRANSFAC professional V10.2 library.

Supplementary Figures

Suppl. Fig.1

A



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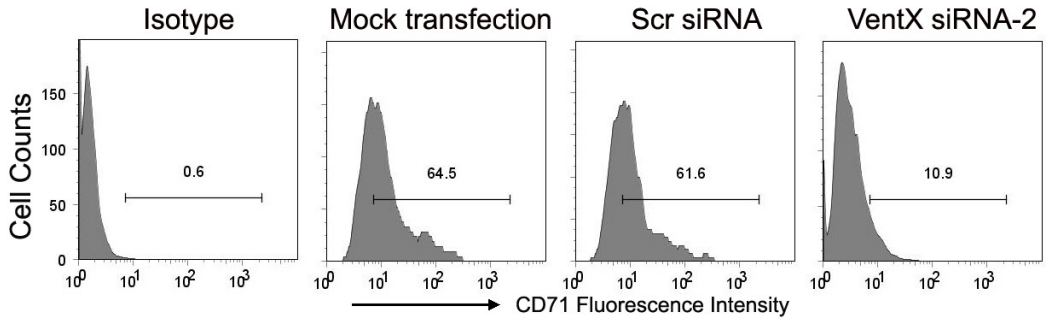

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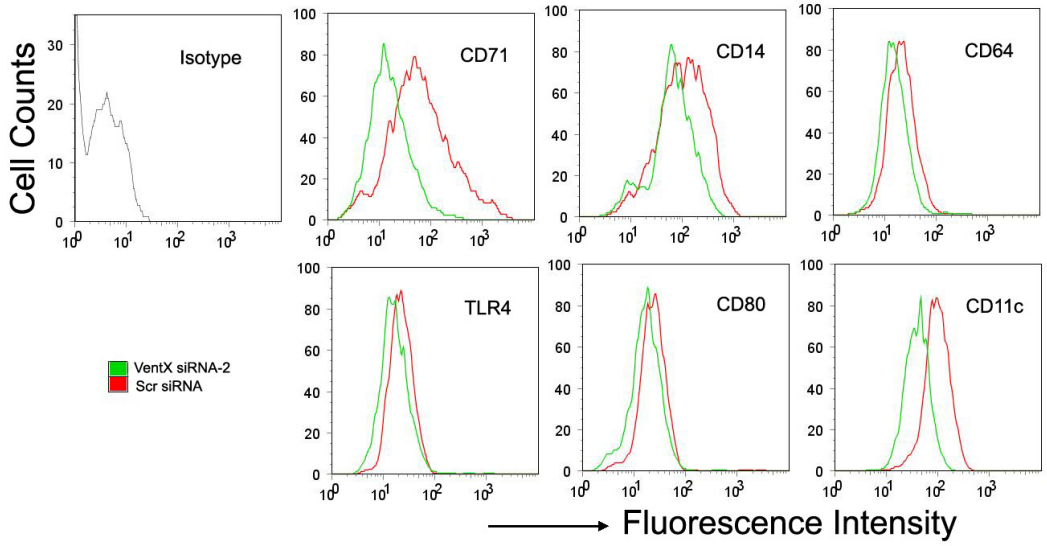
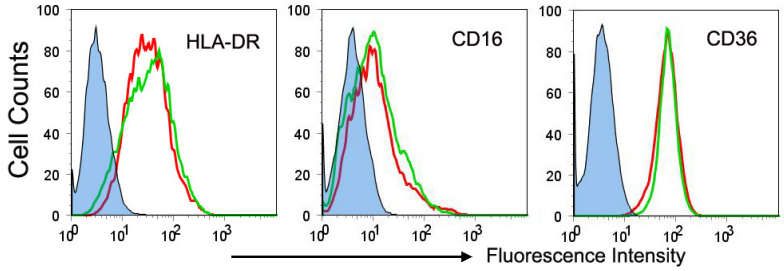
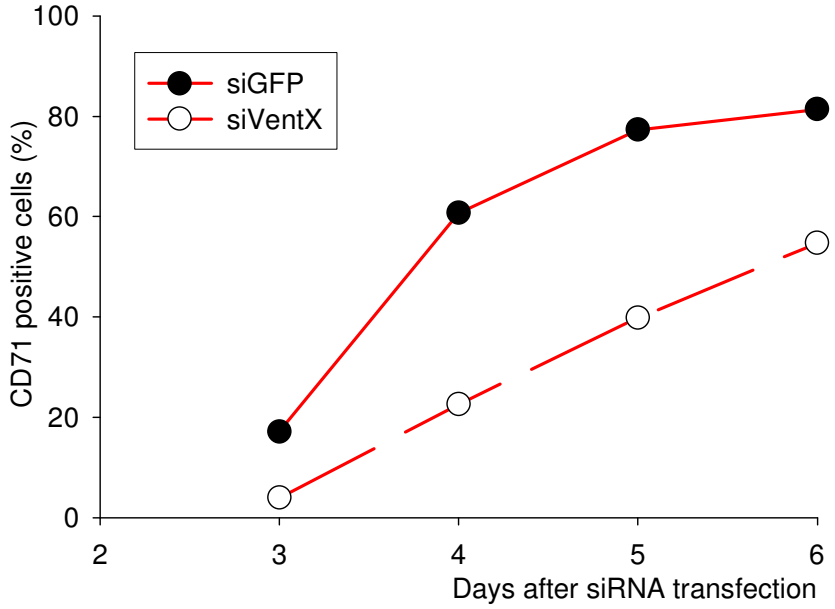
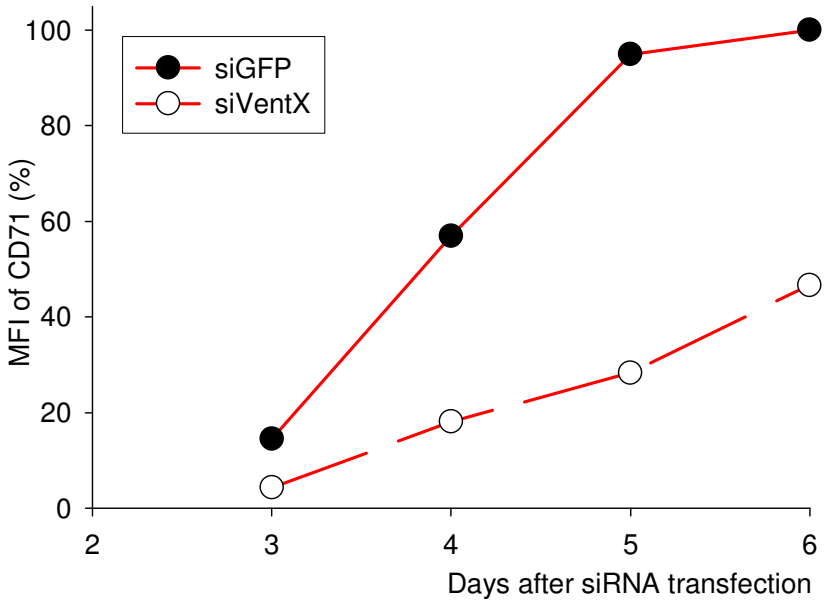


Suppl. Fig.2



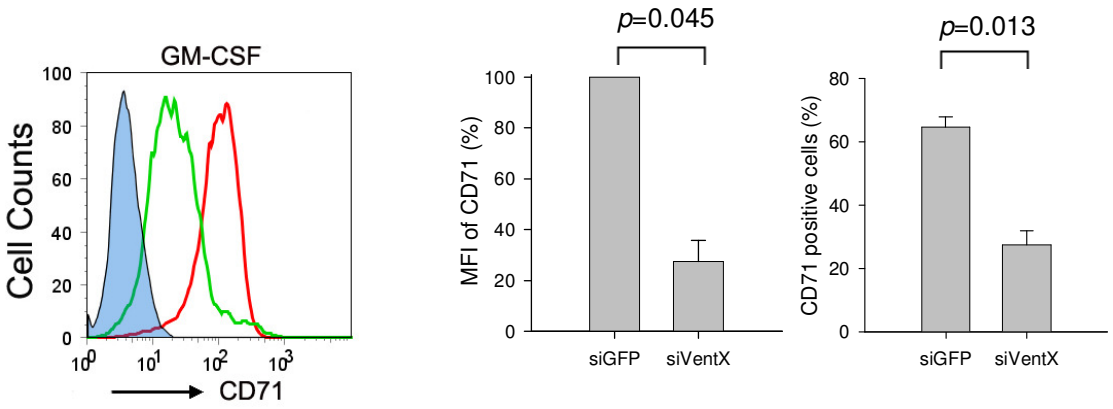
Suppl. Fig. 3


100
80
60
40
20
0
CD71 positive cells (%)
siGFP
siVentX
2
3
4
5
6
Days after siRNA transfection


100
80
60
40
20
0
MFI of CD71 (%)
siGFP
siVentX
2
3
4
5
6
Days after siRNA transfection

Suppl. Fig. 4

A



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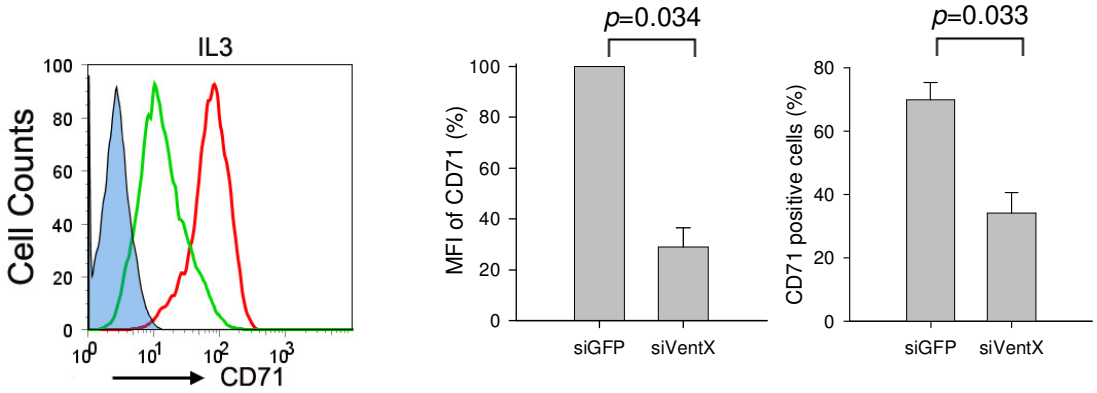

Suppl. Fig. 5

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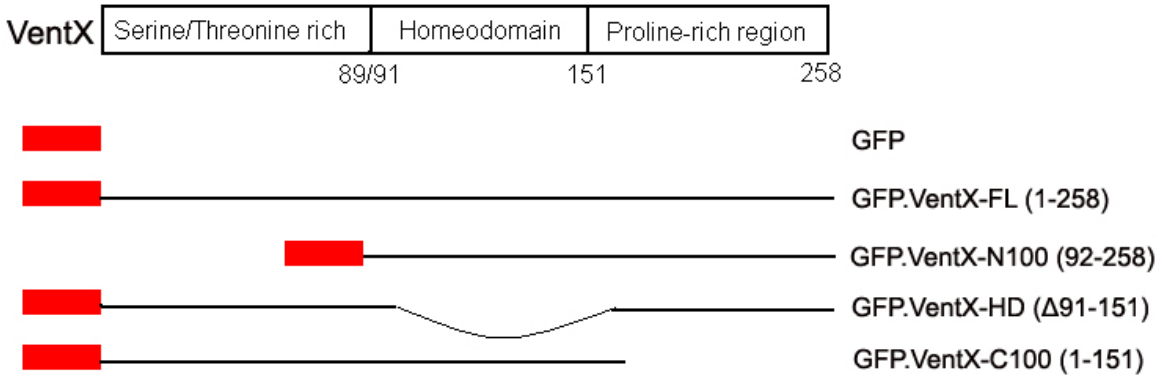

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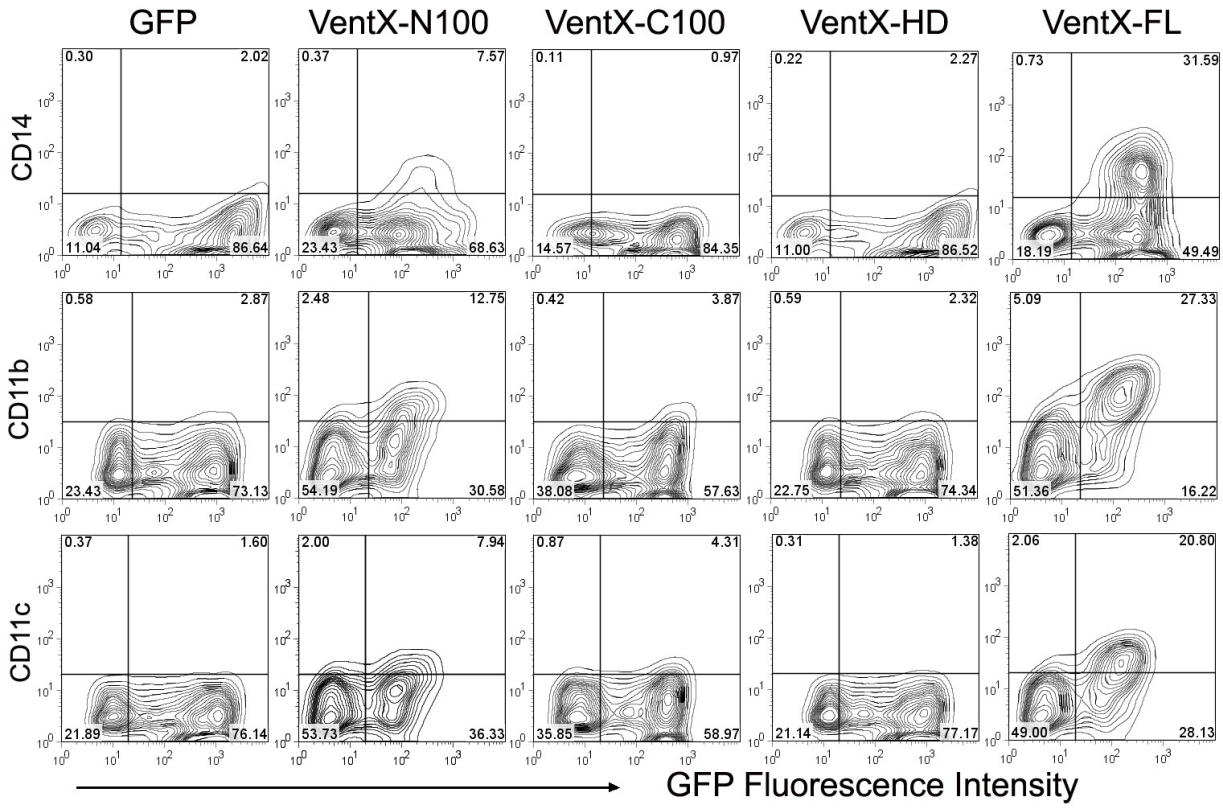

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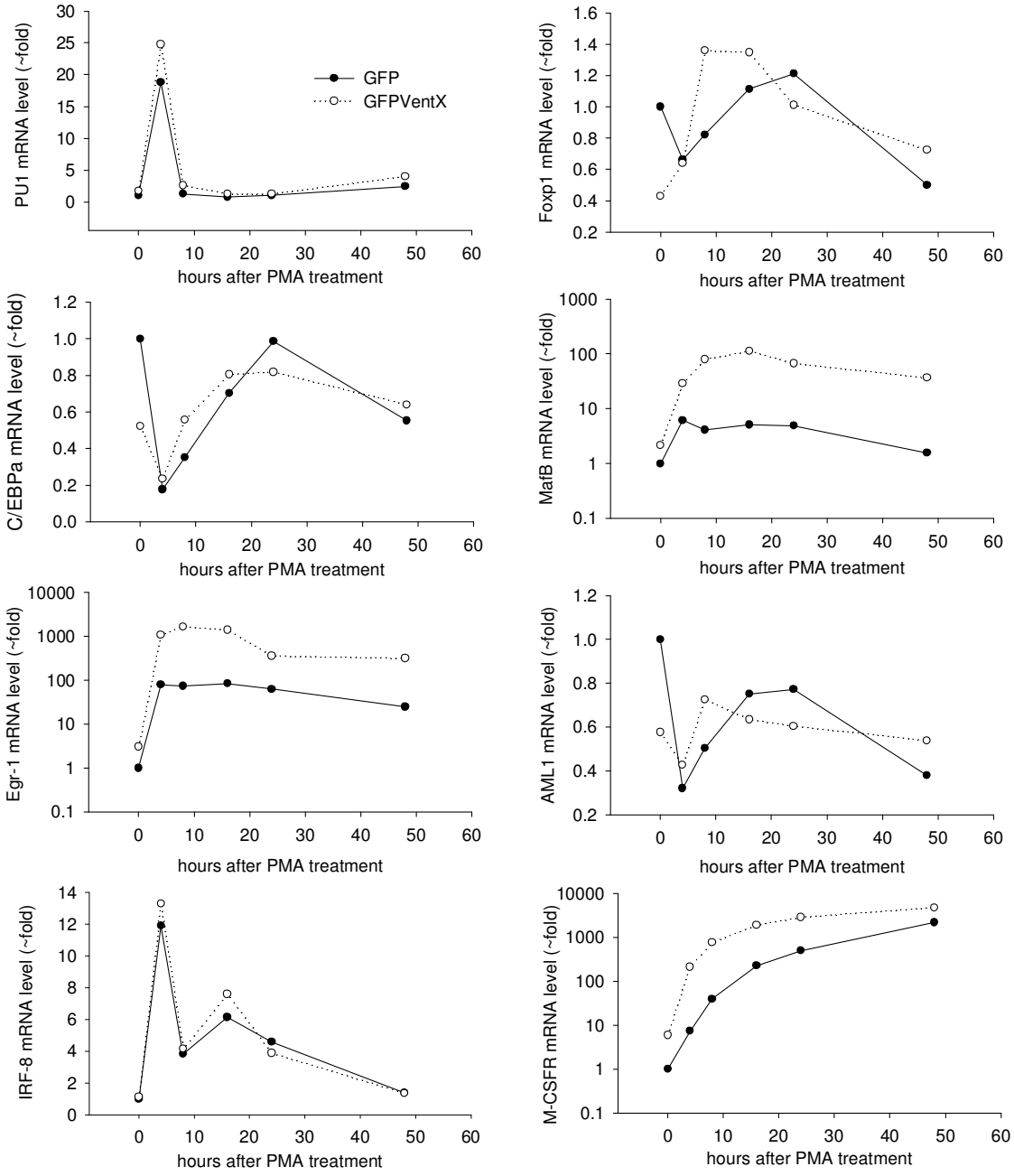
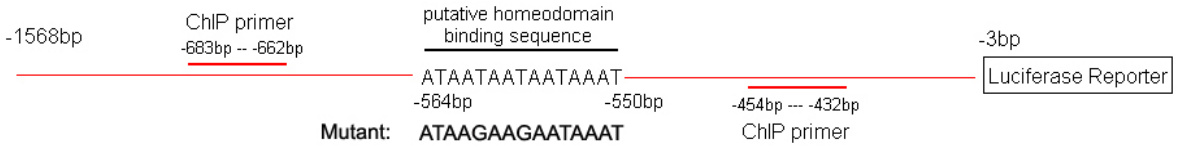

PU1 mRNA level (~fold)
GFP
GFPVentX
hours after PMA treatment
Foxp1 mRNA level (~fold)
hours after PMA treatment
C/EBPa mRNA level (~fold)
hours after PMA treatment
MafB mRNA level (~fold)
hours after PMA treatment
Egr-1 mRNA level (~fold)
hours after PMA treatment
AML1 mRNA level (~fold)
hours after PMA treatment
IRF-8 mRNA level (~fold)
hours after PMA treatment
M-CSFR mRNA level (~fold)
hours after PMA treatment

Suppl. Fig. 7

A



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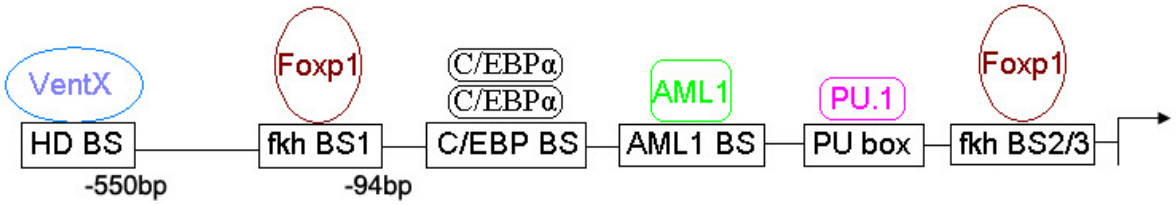
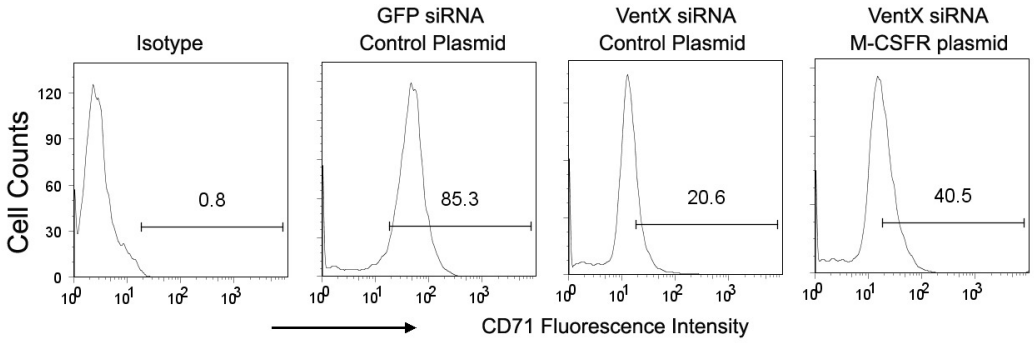
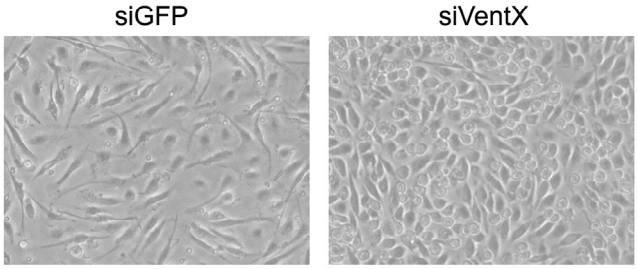


Suppl. Fig.8


Isotype
GFP siRNA
Control Plasmid
VentX siRNA
Control Plasmid
VentX siRNA
M-CSFR plasmid
Cell Counts
120
90
60
30
0
0.8
85.3
20.6
40.5
10^0 10^1 10^2 10^3
CD71 Fluorescence Intensity

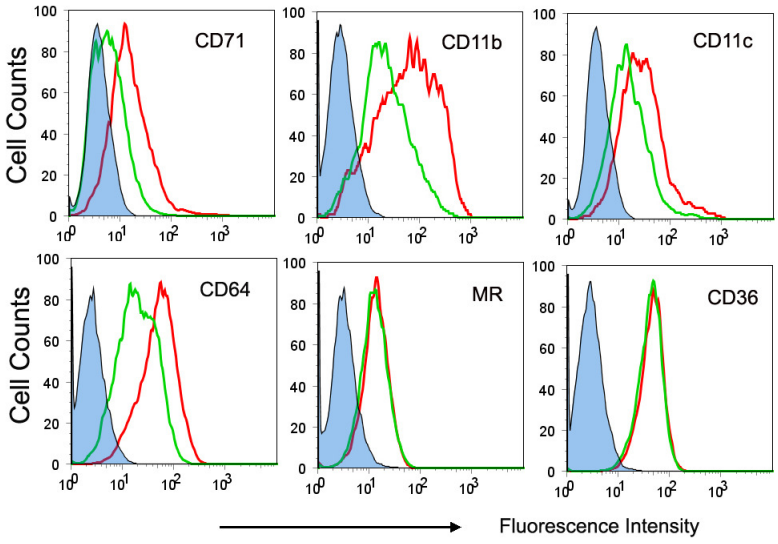
Suppl. Fig. 9

A



	siGFP	siVentX
Adhesion	86.3 ± 6.4%	33.8 ± 4.9%
Morphology change	67.8 ± 6.3%	6.6 ± 2.4%

B



Suppl. Fig. 10

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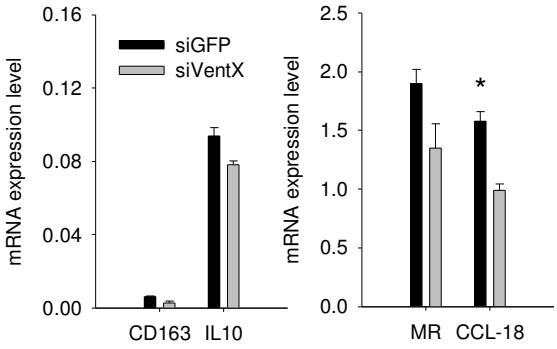

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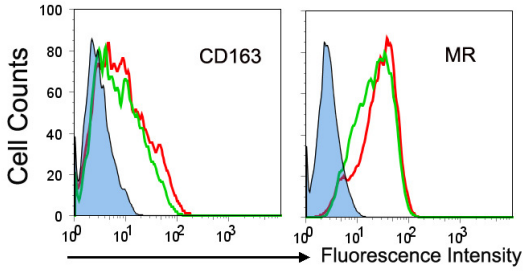
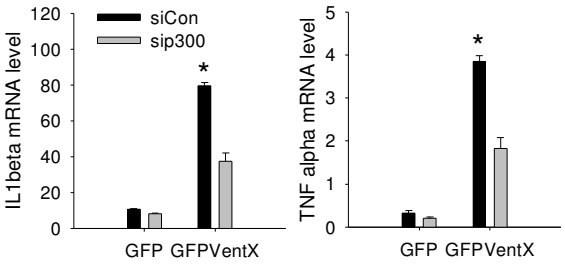
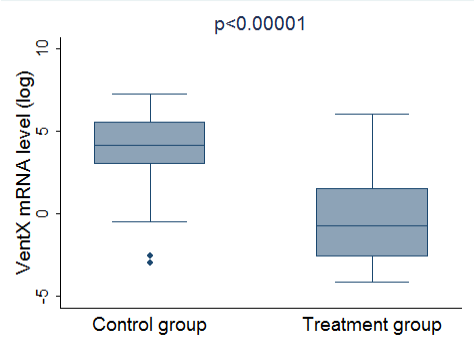


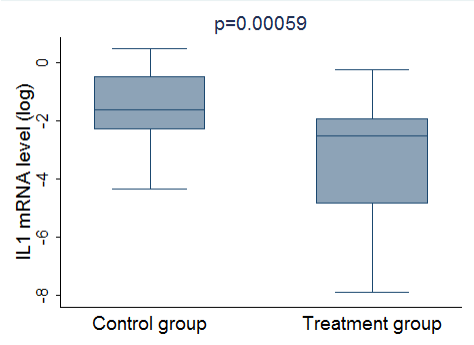
Suppl. Fig. 11

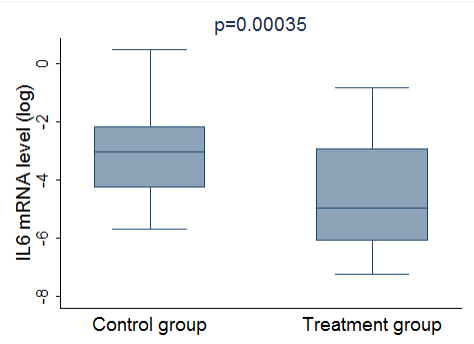

siCon
sip300
IL1beta mRNA level
120
100
80
60
40
20
0
*
GFP
GFPVentX
TNF alpha mRNA level
5
4
3
2
1
0
*
GFP
GFPVentX

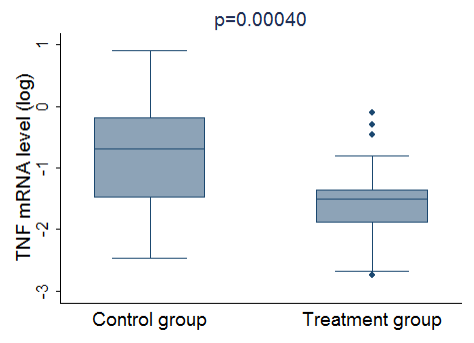
Suppl. Fig. 12

A









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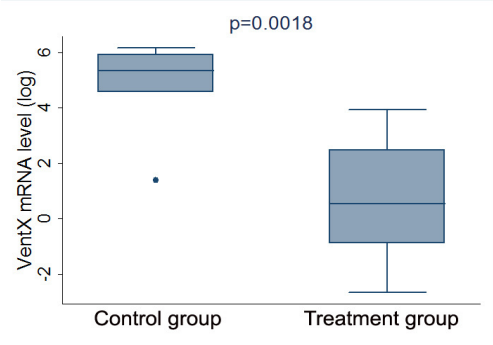
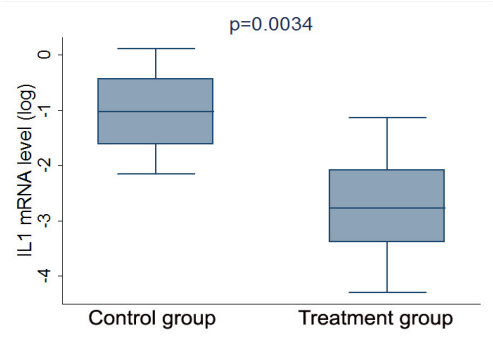
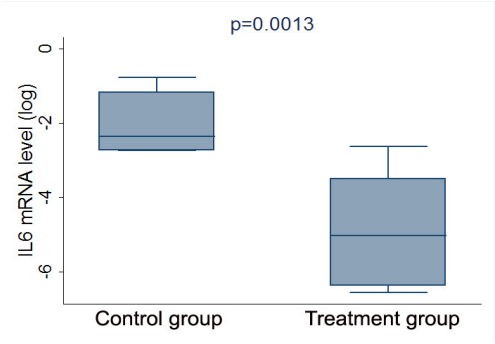
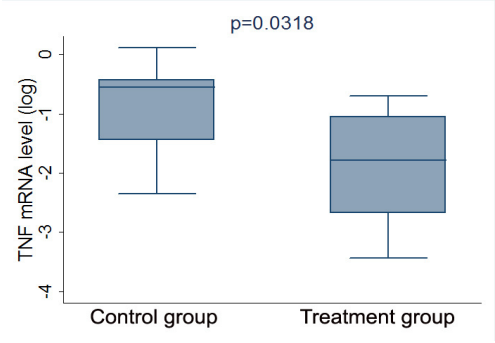


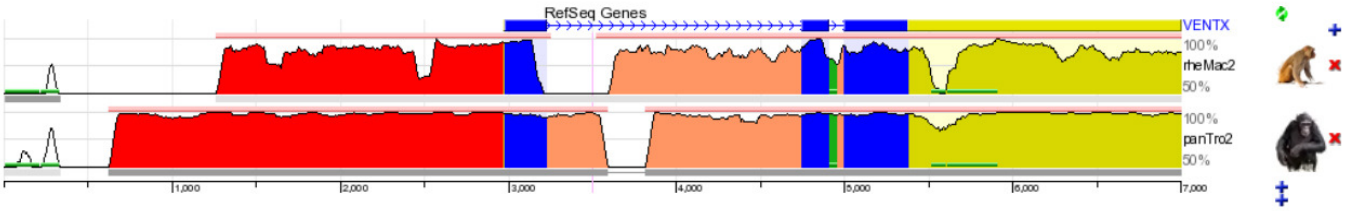






Suppl. Fig. 13

A



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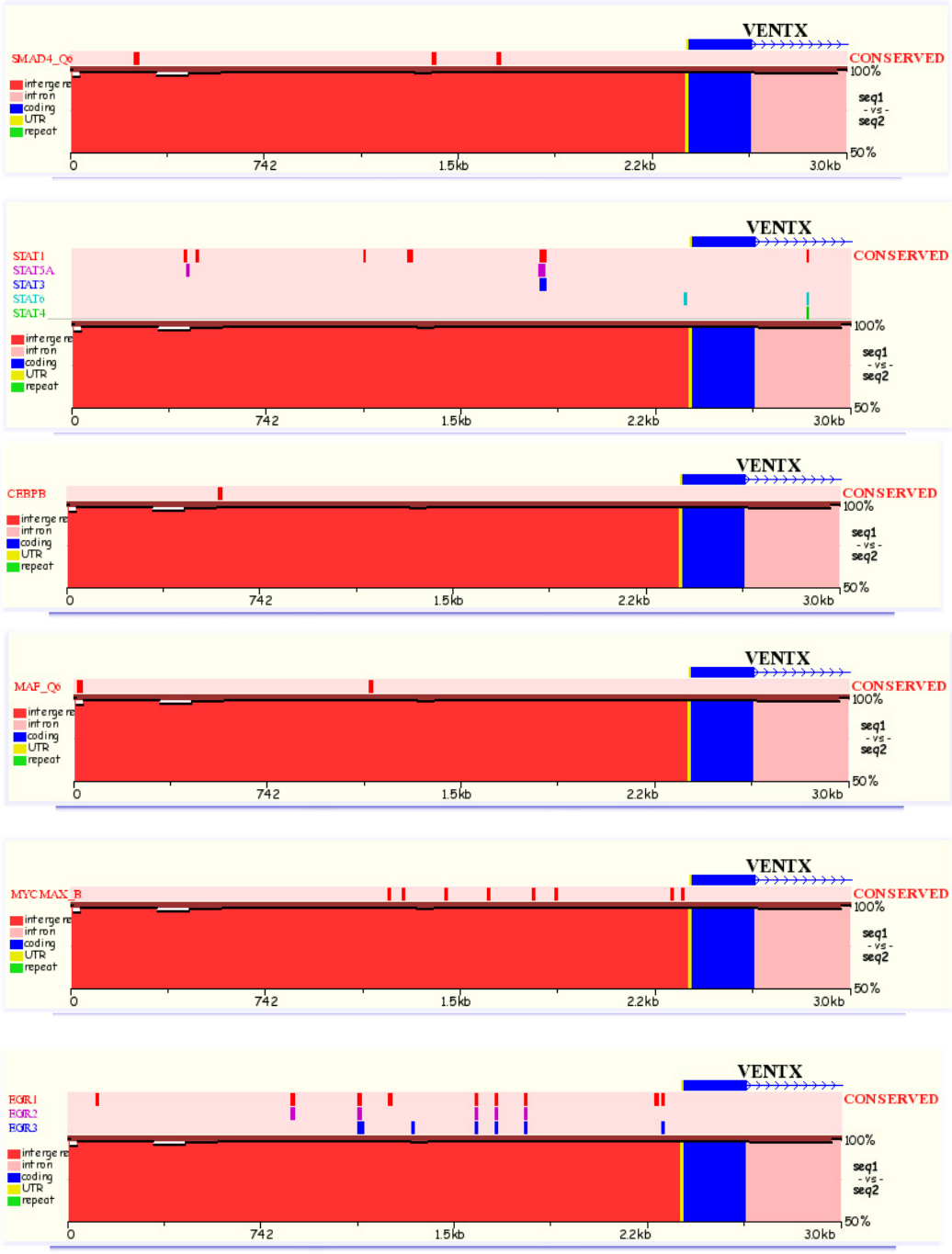
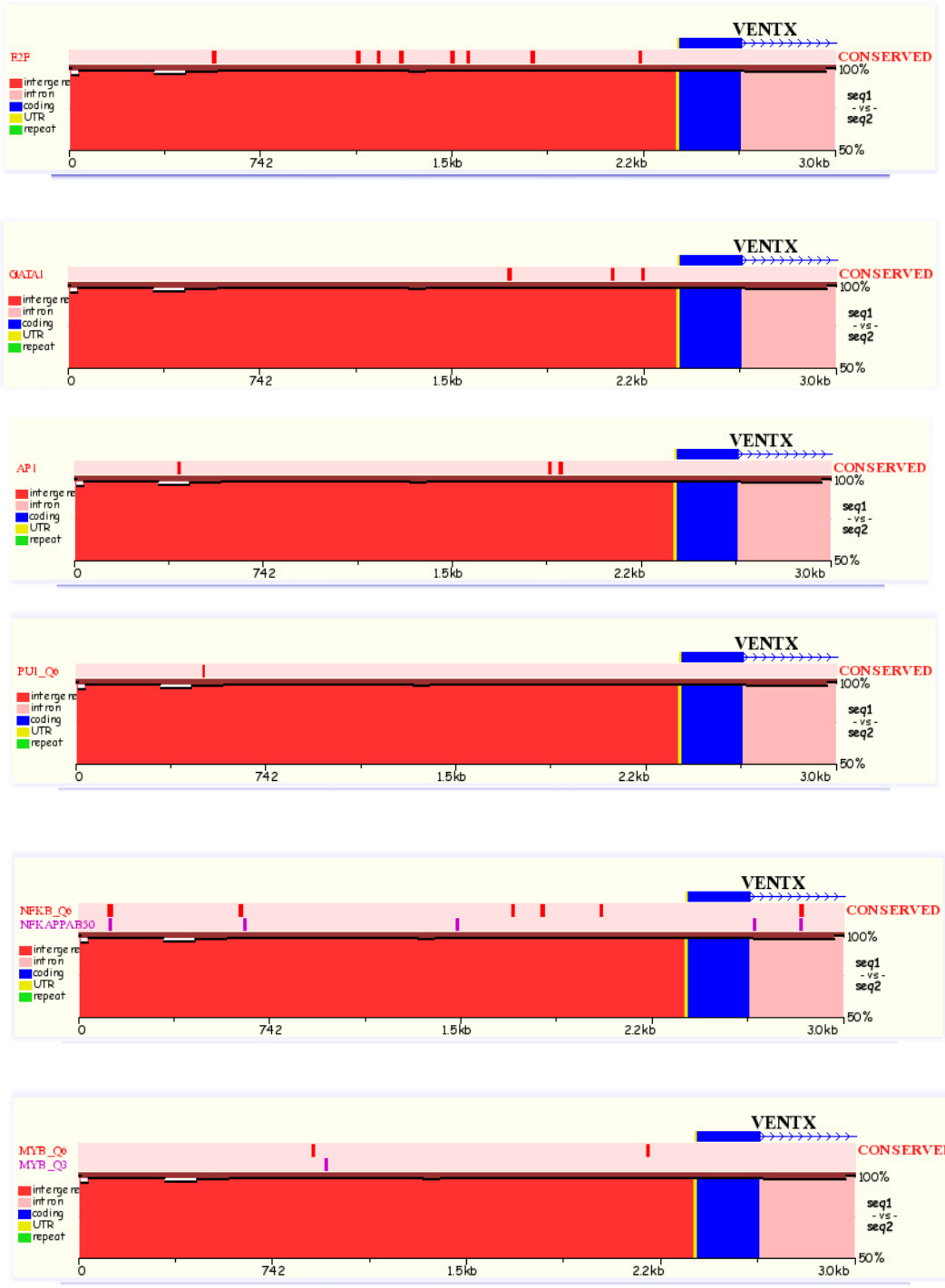


Suppl. Fig. 13 B *Continued:*



Supplementary Table 1

Primer sequences used in this study

	Forward	Reverse
VentX-C*	AAGGCAATTAGGCGCTGCTT	ACAGAACAACTGAGTCCTCCA
VentX-R*	CCGTCAGCATCAAGGAGG	CTGGACCTCTGAGAGCTGC
IL1β	AAGCTGATGGCCCTAAACAG	AGGTGCATCGTGCACATAAG
IL6	GAACTCCTTCTCCACAAGCGCCTT	CAAAAGACCAGTGATGATTTTCACCAGG
TNFα	CGC CAC CAC GCT CTT CTG	GCC ATT GGC CAG GAG GGC
IL8	ATGACTTCCAAGCTGGCCGT	CCTCTTCAAAAACTTCTCCACA
M-CSF	GTACTGTAGCCACATGATTGG	CTGGAGCATTCAGCAAAGCTG
M-CSFR	CGGTGCAGAGCCTGCTGACTG T	ACAGGCTCCCAGAAGGTTGACG
IL12p35	GCGCGCAGCCTCCTCCTTG	TGGAGGCCAGGCAACTCCCA
IL12p40	GCAGAGGCTCTTCTGACCCCCA	AGCTGACCTCCACCTGCCGA
TLR4	AAGCCGAAAGGTGATTGTTG	CTGAGCAGGGTCTTCTCCAC
CD14	CGCAACACAGGAATGGAGAC	CCAGCGAACGACAGATTGAG
MD-2	GAATCTTCCAAAGCGCAAAG	AGGATGACAAACTCCAAGCA
IFNGR1	CATCACGTCATACCAGCCATTT	CTGGATTGTCTTCGGTATGCAT
IFNGR2	CAAGGACAGCTCACCAAAGGATGACG	CAGCTCCGATGGCTTGATCTCTTCCA
JunB	ATGGAACAGCCCTTCTACCACG	AGGCTCGGTTTCAGGAGTTTG
JunD	GTCTACGCGAACCTGAGCAGCTA	CTCGTCCTTGAGCGCAGCCAGGC
c-Jun	TCGACATGGAGTCCCAGGA	GGCGATTCTCTCCAGCTTCC
c-Fos	TGCCTCTCCTCAATGACCCTGA	ATAGGTCCATGTCTGGCACGGA
fosB	AGCAGCAGCTAAATGCAGGA	TTTTGGAGCTCGGCGATCT
RelA	GAAGAAGAGTCCTTTCAGCG	GGGAGGACGTAAAGGGATAG
RelB	GCAGCGAGCCATTGCCTTTC	GGTCCAGCATGGTGAAGAGTGT
c-Rel	CGAACCCAATTTATGACAACCG	TTTTGTTTCTTTGCTTTATTGCCG
NFκB1	TCCACAAGGCAGCAAATAGA	GGGGCATTTTGTTGAGAGTT
NFκB2	TTCTGAAGGCTGGT CTGAC	AGTGAGGTCAAGAGGCGTGT
β-actin	GCAAAGACCTGTACGCCAAC	CTAGAAGCATTTGCGGTGGA
CD163	ACATAGATCATGCATCTGTCATTTG	CATTCTCCTTGGAATCTCACTTCTA
CCL18	AGCTCTGCTGCCTCGTCTAT	CCCACTTCTTATTGGGGTCA
IL10	GATCCAGTTTTACCTGGAGGAG	CCTGAGGGTCTTCAGGTTCTC
MR	CGAGGAAGAGGTTCGGTTCACC	GCAATCCCGGTTCTCATGGC
p21	AAACTTTGGAGTCCCCTCAC	AAAGGCTCAACACTGAGACG
AML1	ACAGAGACATTGCCAACCAT	CAGGACATTTGAGTGGAACC
Foxp1	AGATATTGCGCAGAACCAAG	CGCACAAAACACTTGTGAAG
C/EBPa	CTAGGAACACGAAGCACGAT	ATGGTGGTTTAGCAGAGACG
Egr-1	TGACCGCAGAGTCTTTTCCT	TGGGTTGGTCATGCTCACTA
HIF-1	GCTGATTTGTGAACCCATTC	AAATTGAGCGGCCTAAAAGT
Stat6	CTAGAGGCCAGGGATAGAGG	CAGCCCTTGTACTTTTGCAT
MafB	GAGAGACGCCTACAAGGTCA	CTCGCTCAAGTCAAACAGGT
PU1	CAAGGTTCCCTCTTGTCAGA	AAAGGGGCAGCAGAATAGTT
KLF2	GAAAAGACCACGATCCTCCT	GAACCAGGTAGCCCAAAAAT
KLF4	ATATGACCCACACTGCCAGA	CCCCTTGGCATTTTGTAAGT
p300	CCAGCAAAAGAAAGGCAAAC	AGTGAAGACCGAGCGAAGCA
IRF8	CCAGGACTGATTTGGGAGAA	AATGGAGGCATCCACTTCCT
c-Myc	CAGCTGCTTAGACGCTGGATT	GTAGAAATACGGCTGCACCGA

C*: conventional PCR; R*: real-time PCR