SUPPLEMENTARY METHODS

Immunocytochemistry. Polymorphonuclear neutrophils (PMNs) were isolated from peripheral blood of donors by sedimentation of red blood cells in Plasmagel (ZeptoMetrix), followed by Ficoll-Hypaque density gradient centrifugation (Sigma). PMNs were cytocentrifuged on glass slides and treated with cytoskeletal buffer (CSK:100mM NaCl; 300mM sucrose; 3mM MgCl2; 10mM PIPES, pH 6.8) for 30 seconds; CSK, 0.5% Triton X-100 for 30 seconds; and CSK for 30 seconds. After CSK treatment, PMNs were fixed with 4% paraformaldehyde/PBS. Slides were incubated in blocking buffer (PBS, 5% heat inactivated goat serum, 0.2% Tween, 0.2% Fish gelatin (Sigma G-7765)) at 37^{0}C for 30 minutes. Slides were incubated with primary antibodies, rabbit anti-H3K27me2,3 [1,2] or rabbit anti-H3K9me2 antibody (Upstate), and followed by secondary antibody, FITC-goat anti-rabbit IgG (Molecular Probes). Coverslips were mounted with Vectashield (Vector Laboratories, Inc.) containing DAPI to stain nuclei. Cells were viewed with 100x oil objective using Lieca DMLB upright microscope (Lieca Microsystems Wetzlar GMBH). Images were captured with SPOT RT Slider Camera and SPOT Software version 4.5 (Diagnostic Instruments Inc.). The 8-bit grayscale images were pseudo-colored with Software version 4.5. Adobe Photoshop version 7.0.1 was used to adjust the dynamic range of pseudo-colored images to the same values and to merge images.

Methyl Specific PCR. Briefly, 500ng genomic DNA that was digested with EcoRI was bisulfite-modified using EZ DNA Methylation-Gold assay (D5005, Zymo Research). Bisulfite-modified DNA was used as a template for PCR with PR3 primers designed for methyl specific PCR with Methyl Primer Express v1.0 (Applied Biosystems), and listed in Supplementary Table 4. PCR conditions with primers specific for methylated DNA were as follows: 95^{o}C 5 min, 40 cycles of 94^{o}C for 30 s, 58^{o}C for 1 min, and 72^{o}C for 1 min, final extension at 72^{o}C for 7 min. PCR conditions with primers specific for unmethylated DNA were as follows: 95^{o}C 5 min, 40 cycles of 94^{o}C for 30 s, 53^{o}C for 1 min, and 72^{o}C for 1 min, final extension at 72^{o}C for 7 min. NIH ImageJ 1.30v was used to quantitate the mean gray scale intensities corresponding to each PR3 PCR product, corrected by subtracting background gray scale values for each lane. The percentage of unmethylated DNA was calculated by dividing corrected mean intensity values for unmethylated product by the sum of the corrected mean intensity for methylated and unmethylated product. The percentage of unmethylated PR3 DNA is listed in Supplementary Figure 5 legend.

DNA methylation. DNA methylation at the *PR3*, *MPO* and *RUNX3* was determined using the SA Biosciences Methyl-Profiler^{TM} DNA Methylation assay (MEA-03). Briefly, 50-100 ng of purified DNA from total leukocytes was used in a mock restriction enzyme digest, DNA methylation sensitive restriction enzyme digest, DNA methylation dependent restriction enzyme digest, and double digest. After an overnight incubation at 37^{o}C, the DNA was analyzed by real-time PCR. Ct values were used to determine the relative DNA methylation levels. The

amount of starting DNA is proportional to the raw Ct value that results from the real-time PCR of the mock digested DNA. The amount of DNA that is resistant to digestion is determined from the Ct value from the real-time PCR of the double digest, Ct_{Mds}. All samples had a ΔCt (Ct double, Ct_{Mds} – Ct mock, Ct_{Mo}) corresponding to greater than 95% complete digestion. The amount of hypermethylated DNA is calculated from the Ct value from the real-time PCR reaction of the DNA digested with methylation sensitive restriction enzyme mix, Ct_{Ms}, i.e. if the DNA is hypermethylated then the DNA is resistant to the methylation sensitive restriction enzyme and available for PCR. The amount of unmethylated DNA is calculated from the Ct value from the real-time PCR reaction of the DNA digested with methylation dependent restriction enzyme mix, Ct_{Md}, i.e. if the DNA is unmethylated then the DNA is resistant to digestion with the methylation dependent restriction enzyme and available for PCR. Intermediately methylated DNA exists when the sum of the hypermethylated and unmethylated DNA is less than 100%, and likely represents a mixture of DNA molecules with methylation at different CpG dinucleotides. Intermediately methylated DNA is detected when the ΔCt (Ct_{Ms} - Ct_{Mo}) and ΔCt (Ct_{Md} - Ct_{Mo}) are both greater than 1. This situation reflects the condition where some of the molecules are digested by the methylation sensitive restriction enzyme and some of the molecules are digested by the methylation dependent restriction enzyme. Since the DNA is not completely resistant to either enzyme the sample is defined as containing intermediately methylated DNA. This is distinct from samples where a fraction of the DNA molecules are unmethylated and the remaining fraction is hypermethylated, or the reciprocal. In this case either the ΔCt (Ct_{Ms} - Ct_{Mo}) or the ΔCt (Ct_{Md} - Ct_{Mo}) would be less than 1. For instance, if less than 50% of the molecules were unmethylated, then less than half of the DNA molecules would be digested with the methylation sensitive restriction enzyme and more than half of the original DNA molecules would be available as templates for PCR. Less than two fold difference in starting template would result in a ΔCt (Ct_{Ms} - Ct_{Mo}) of less than 1.

The data in Table 1 is presented as the mean$\pm$standard deviation of the percentage methylated (hypermethylated plus intermediately methylated) and unmethylated DNA. The methylation status at the CpG islands examined in *PR3* and *MPO* was fully described by hypermethylated and unmethylated categories. The methylation status at *RUNX3* also contained intermediately methylated DNA at the *RUNX3* CpG island, and is reported as the sum of hypermethylated and intermediately methylated DNA in Table 1. The density of CpG dinucleotides in the *RUNX3* promoter is greater than the density of CpG dinucleotides in amplicons interrogated at *PR3* and *MPO*. Transcriptional repression mediated by DNA methylation of *RUNX3* may occur with methylation at a fraction of the CpG dinucleotides at the *RUNX3* promoter, resulting in a mixture of DNA molecules with methylation at different CpG dinucleotides, i.e. intermediately methylated DNA. It remains to be determined if an intermediate level of DNA methylation at the *RUNX3* promoter is sufficient for transcriptional repression; however, the level of unmethylated DNA at the *RUNX3* promoter, which is determined directly with the Methyl-Profiler^{TM} DNA Methylation assay, is elevated

in healthy controls compared to ANCA patients. In Supplementary Figure 4b the average percentages of the three DNA methylation categories are shown for *RUNX3*.

REFERENCES

1. Hamer, K.M. et al. A panel of monoclonal antibodies against human polycomb group proteins. *Hybrid Hybridomics* **21**, 245-52 (2002).
2. Montgomery, N.D. et al. The murine polycomb group protein Eed is required for global histone H3 lysine-27 methylation. *Curr Biol* **15**, 942-7 (2005).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Histone methylation marks, H3K27me2,3 and H3K9me2, are present in neutrophils from ANCA vasculitis patients. (**a-f**) Top six panels show neutrophils from a healthy control (panel **a-c**) and an ANCA vasculitis patient (panel **d-f**) stained with anti-H3K27me2,3 antibody. Merged images of dapi stained nuclei (**a,d**) and immunofluorescence (**b,e**) are shown in **c** and **f**. (**g-l**) Bottom six panels show neutrophils from a healthy control (panel **g-i**) and an ANCA vasculitis patient (panel **j-l**) stained with anti-H3K9me2 antibody. Merged images of dapi stained nuclei (**g,j**) and immunofluorescence (**h,k**) are shown in **i** and **l**.

Supplementary Figure 2. Methylation of histone H3K9 is not different at PR3 and MPO between ANCA vasculitis patients and healthy controls. (**a-b**) Quantitative ChIP analysis for H3K9me2 enrichment on chromatin isolated from neutrophils of healthy controls (open circles, n=23) and ANCA vasculitis patients (filled diamonds, n=15) is shown for genomic regions of *PR3* (**a**) and *MPO* (**b**). Levels of H3K9me2 immunoprecipitated chromatin are reported as a percent of input DNA.

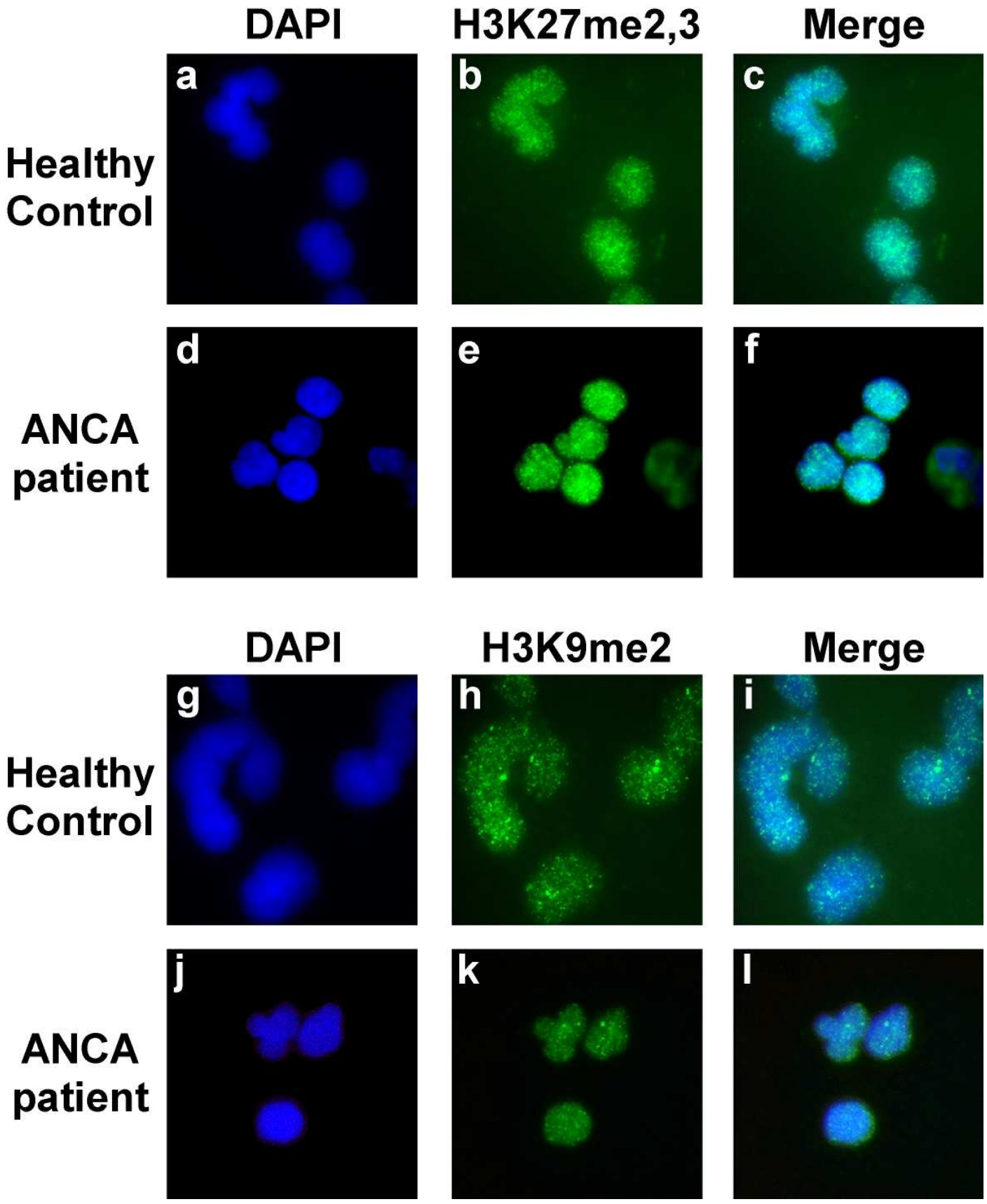
Supplementary Figure 3. Stratified *PR3*, *MPO*, *JMJD3*, and *RUNX3* relative mRNA levels. Relative mRNA levels for *PR3* (**a**), *MPO* (**b**), *JMJD3* (**c**), and *RUNX3* (**d**) are shown for healthy controls (blue diamonds, n=49) ANCA patients in remission (yellow diamonds, n=30), and active ANCA patients (red diamonds, n=67). Patients were separated into remission or active based on BVAS score. ANCA patients with a BVAS $\leq$ 1 were classified as remission; ANCA patients with a BVAS >1 were classified as active. Mean and median values for relative mRNA expression of *PR3*, *MPO*, *JMJD3* and *RUNX3* are listed for each class beneath the charts. *P*-values of two-tailed ttests between healthy controls and all ANCA patients (*****) and healthy controls and ANCA patients with active disease (#) are listed above each chart.

Supplementary Figure 4. DNA methylation analysis. (**a**) Methyl specific PCR analysis of *PR3* (CpG island at Chr 19:843469-844085, Bld37). Top panel is an ethidium bromide stained 2% agarose gel with samples from PCR that used primers specific to methylated DNA following bisulfite conversion of genomic DNA. The 134bp PCR product is specific for DNA methylation at *PR3*. Bottom panel is an ethidium stained 2% agarose gel with samples from PCR that used primers specific to unmethylated DNA following bisulfite conversion of genomic DNA. The unmethylated status of *PR3* is indicated by a 137bp PCR product. Lanes 1 and 2 are controls for methylated DNA (lane 1, U937 bisulfite modified genomic DNA) and unmethylated DNA (lane 2, THP-1 bisulfite modified genomic DNA). Lane 3 is no template control (NTC). Lanes 5-8 are PCR samples of bisulfite converted genomic DNA from healthy controls. Lanes 9-12 are PCR samples of bisulfite converted genomic DNA from ANCA patients. NIH ImageJ was used to quantitate the mean gray scale intensity for the methylated and

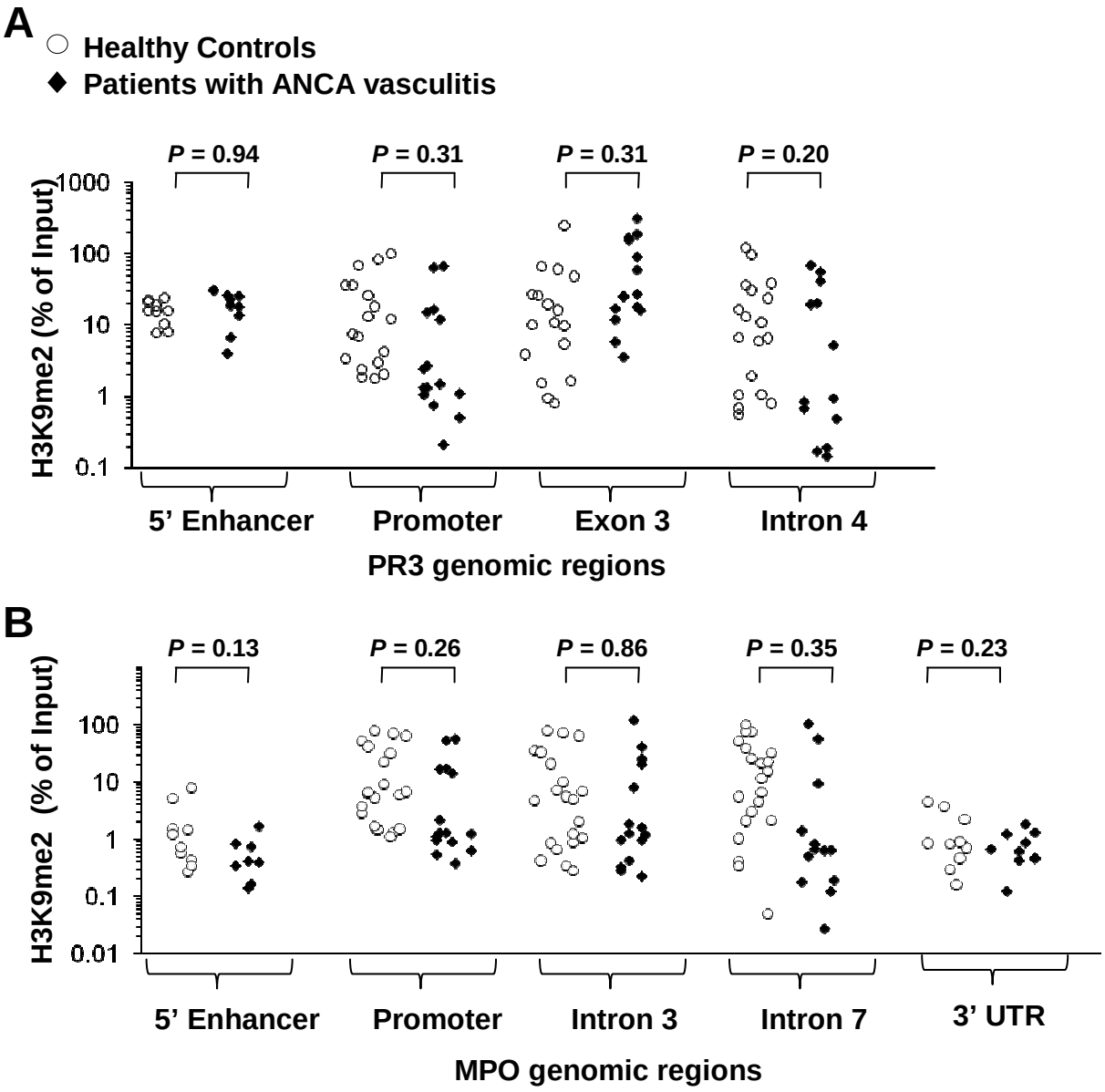
unmethylated specific PCR products and values used to calculate percentage of methylated and unmethylated *PR3* DNA (see Supplementary Methods). The mean unmethylated DNA at *PR3* among 4 healthy controls is 92.6±3.1% and the mean unmethylated DNA at *PR3* among 4 ANCA patients is 91.7±1.9%. A two-tailed ttest (*P*=0.64) indicates percentage of unmethylated DNA at *PR3* is not significantly different between healthy controls and ANCA patients. (**b**) Graphical representation of the mean DNA methylation states (unmethylated; UM, hypermethylated; HM, intermediately methylated; IM) at *PR3*, *MPO*, *RUNX3* measured using methylation sensitive and dependent restriction enzymes followed by quantitative real-time PCR. For both *PR3* and *MPO* the methylation status was fully described by unmethylated and hypermethylated categories. The methylation status at *RUNX3* also contained intermediately methylated DNA. In ANCA patients 76.9±16.2% of the *RUNX3* CpG island examined is hypermethylated, 14.2±18.0% is intermediately methylated, and 8.9±8.8% is unmethylated. In healthy controls 59.5±13.2% of the RUNX3 CpG island is hypermethylated, 15.0±21.5% is intermediately methylated, and 25.5±14.7% is unmethylated. The *P* values of two-tailed ttests between ANCA patients and healthy controls for hypermethylated, intermediately methylated and unmethylated are 0.095, 0.94, and 0.032, respectively.

Supplementary Figure 5. Standard curve for mouse *Mpo* mRNA levels. Real time PCR was used to calculate ΔCt values for mouse *Mpo* mRNA from serial dilutions of mouse *Mpo* positive cells (WEHI). ΔCt values were determined for undiluted (1) WEHI cells, 1:10 dilution (0.1) of WEHI cells with mouse *Mpo* negative cells (mouse embryonic stem cells), 1:100 dilution (0.01), and 1:1000 dilution (0.001), represented by red diamonds. The relative level of mouse *Mpo* mRNA in leukocytes was calculated using the following equation:
$y = -1.3958\ln(x) + 2.1435$. The ΔCt values for *Mpo* mRNA from leukocytes of C57Bl/6 and 129 without anti-MPO antibody, and 129 after anti-MPO antibody are represented by large black diamond, gray diamond and white diamond, respectively.

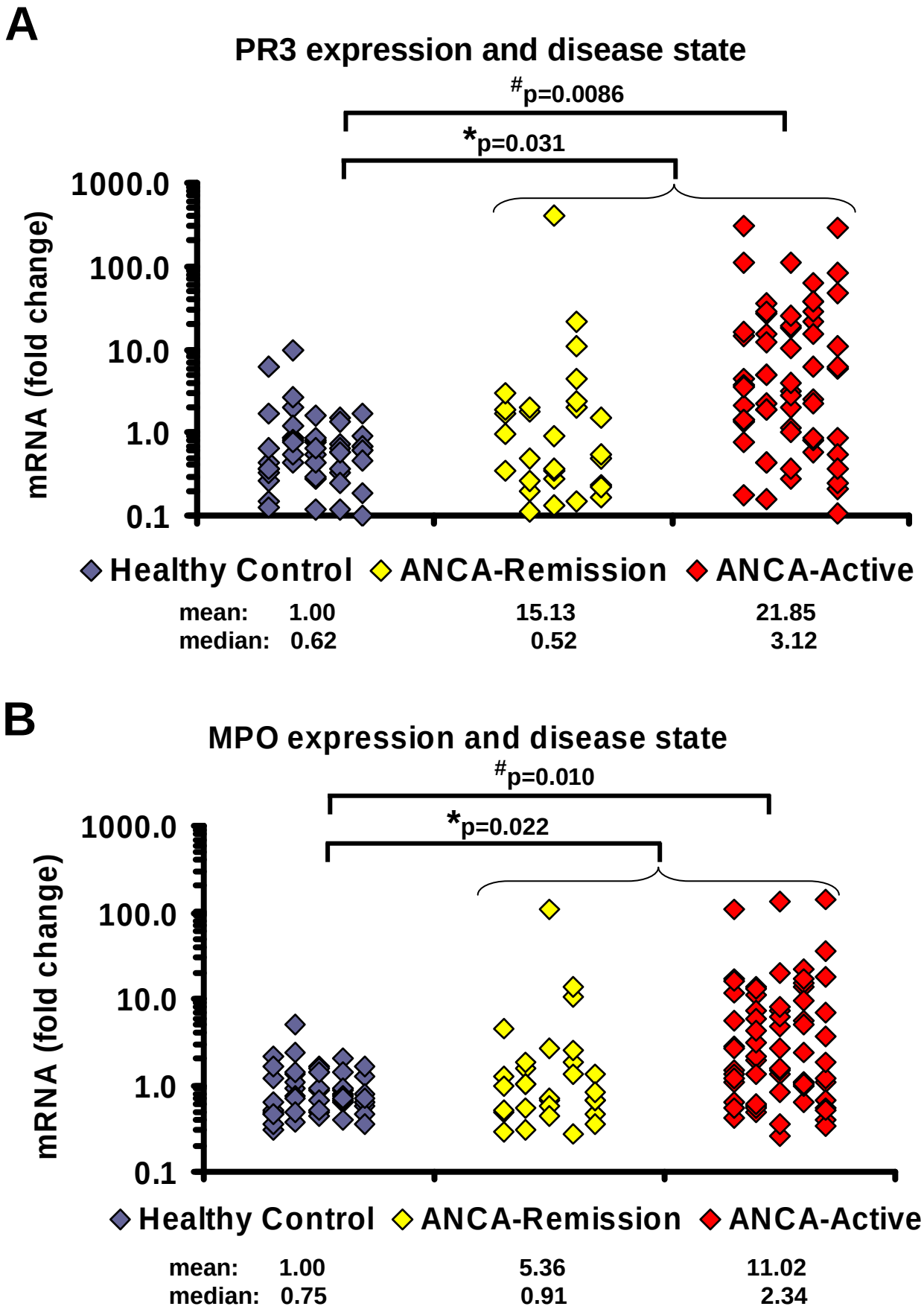
Supplementary Figure 1



Supplementary figure 2

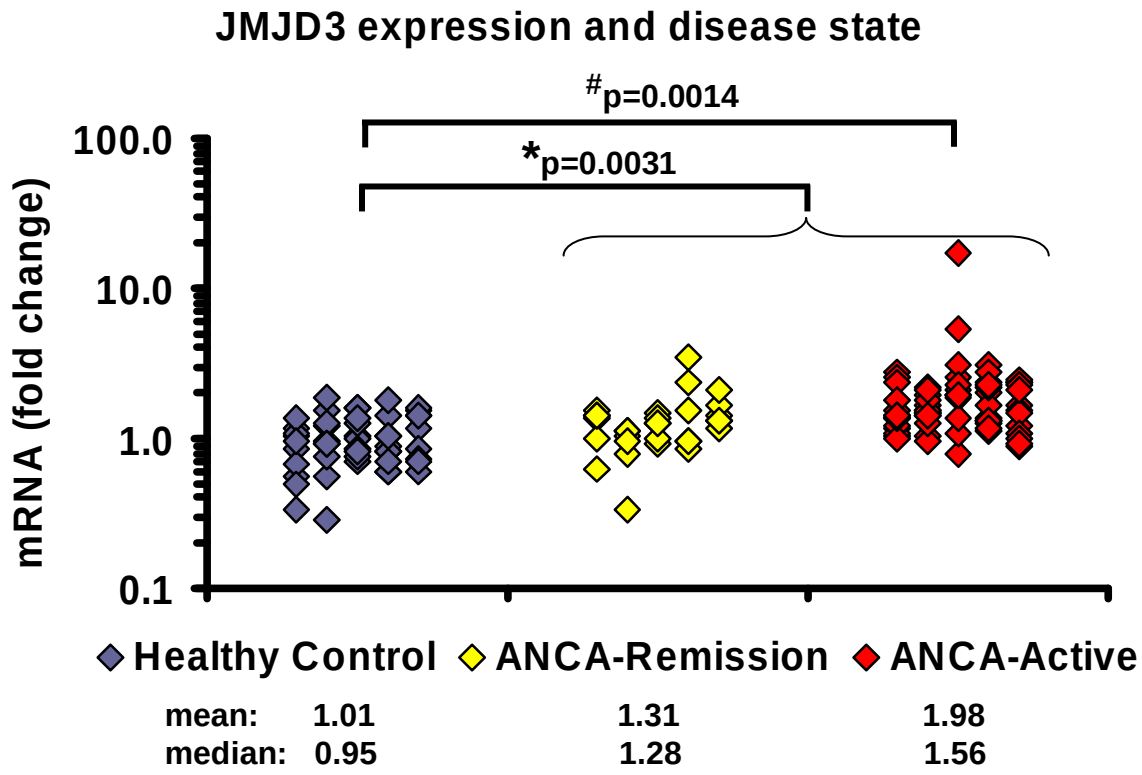


Supplementary figure 3



Supplementary figure 3 (continued)

C



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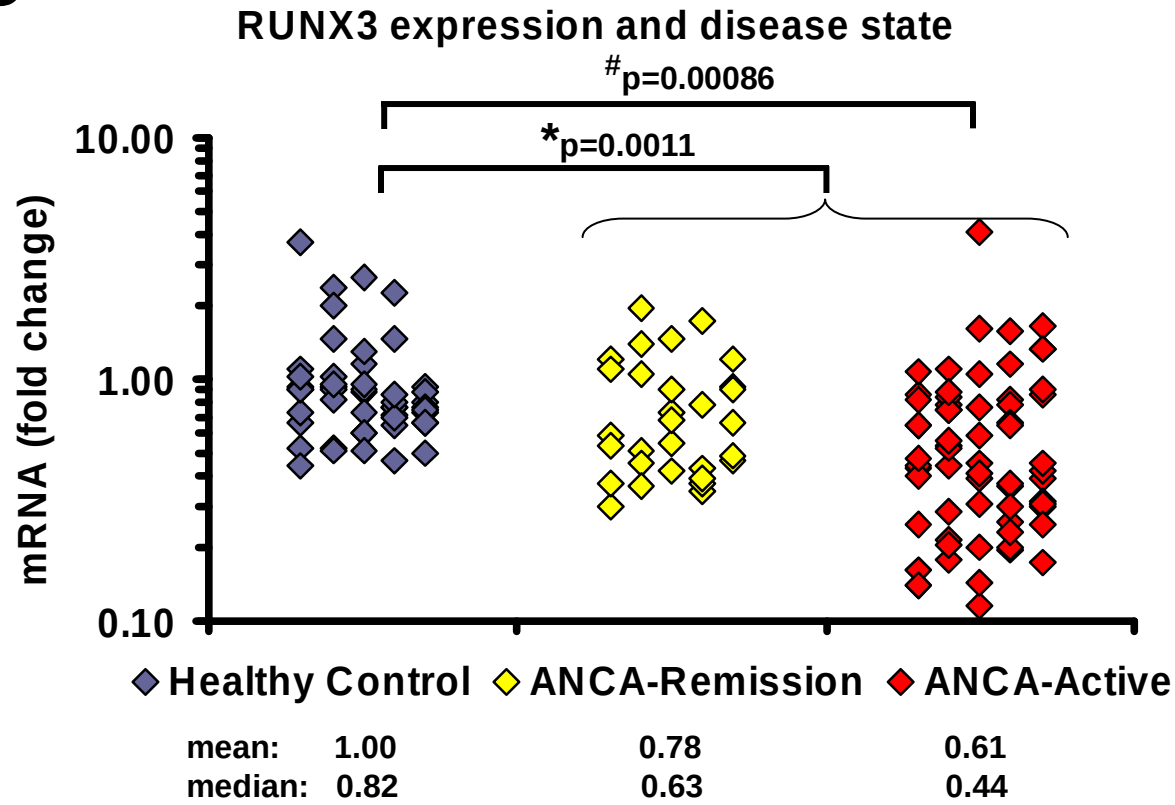

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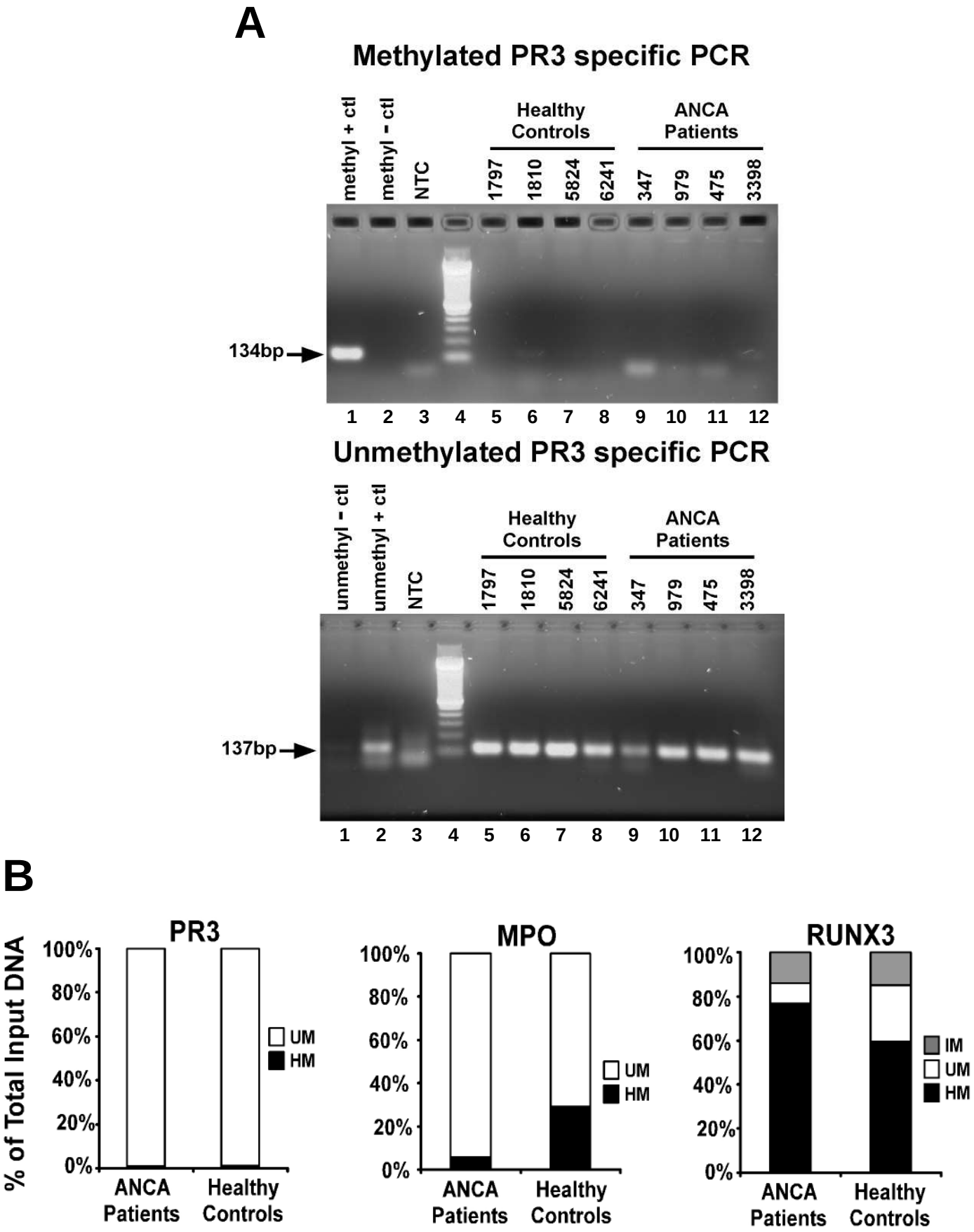
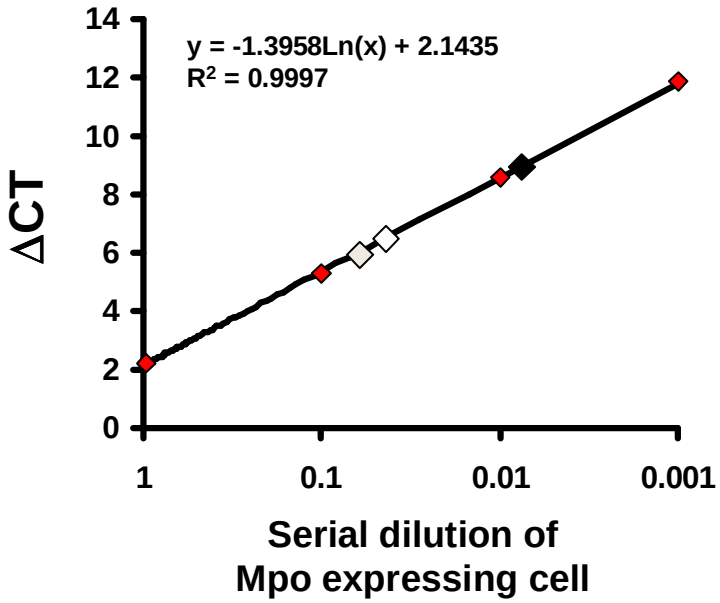


Supplementary figure 5



Supplementary Table 1.
Characteristics of patients with ANCA disease enrolled in the epigenetics study

H3K27me3 level	*Patient*	*Diagnosis*	*Age*	*Sex*	*Race*	*Disease Status*	*ANCA Titer PR3*	*ANCA Titer MPO*	*Scr mg/dl*	*Therapy*
Normal	ANCA 1	WG	40	M	other	Remission	2.8	23.7	0.8	CS
Normal	ANCA 2	WG	32	M	W	Remission	89.7	2.5	1.2	CS,CP,Rituxan
Normal	ANCA 3	WG	43	F	W	Remission	11.8	1.3	0.8	CS,PLEX,HD,MMF
Normal	ANCA 4	MPA	49	F	W	Remission	95.9	3	0.8	CS,AZA
Normal	*ANCA 5	WG	19	F	W	Active	91.3	3	1.5	CS,CP
Low	ANCA 6	WG	63	F	AA	Active	6.8	34.1	2.2	CS,PLEX,HD
Low	ANCA 7	WG	51	M	W	Active	65.3	3.5	1.5	CS,CP
Low	ANCA 8	WG	63	M	W	Active	2.3	22.7	2.2	CS,CP,AZA
Low	ANCA 9	MPA	83	F	other	Active	2.3	60.1	1.7	CS,CP
Low	ANCA 10	WG	59	M	W	Active	29.7	2.4	2.9	CS,MMF
Low	ANCA 11	WG	49	M	W	Active	106.6	2.5	7.1	CS,MMF
Low	ANCA 12	MPA	72	M	W	Active	3.8	122.7	2.8	(-)
Low	ANCA 13	WG	59	M	W	Active	2.9	75.9	0.8	CS,CP,PLEX
Low	ANCA 14	MPA	28	M	W	Active	136.6	4.2	5.0	CS,CP,MMF,Rituxan
Low	§ANCA 15	MPA	31	F	W	Remission	2.8	69.4	1.7	(-)

*Based on active disease status, this patient would be predicted to have low levels of H3K27me3
§ Based on remission disease status, this patient would be predicted to have normal levels of H3K27me3.

CS, corticosteroids; CP, cyclophosphamide; MMF, mycophenolate mofetil; AZA, azathioprine; PLEX, plasma exchange; HD, hemodialysis

Supplementary Table 2.
Demographics of subject populations

	Healthy Controls			***ANCA Patients***		
Analytical method	Affy ® array	TaqMan® PCR	Chromatin IP	Affy® array	TaqMan® PCR	Chromatin IP
Unique donors (n)	16	49	23	21	69	15
Samples (n)	16	49	23	25	97	15
Sex (% male)	61	51	48	55	52	64
Race (% W/AA)*	82/4	74/18	74/0	82/18	81/15	77/9
Age (years)	29±7	42±17	33±14	48±19	50±17	47±17
BVAS[¥]	NA[§]	NA	NA	10.4±8.0	6.7±7.4	9.6±8.6

*W/AA: Caucasian/African American
[¥]BVAS: Birmingham Vasculitis Activity Score
[§]NA: not applicable

Supplementary Table 3.
RNA Primers (Integrated DNA Technologies, INC. Coralville, IA, USA)

PR3	Forward Primer	5'-TGT CAC CGT GGT CAC CTT CTT-3'
	Reverse Primer	5'-CCC CAG ATC ACG AAG GAG TCT AT-3'
	Probe	FAM-TTG CAC TTT CGT CCC TCG CCG-TAMRA
PR3	Forward Primer 394	5'-CTG AGC AGC CCA GCC AAC CT-3'
	Reverse Primer 624	5'-GAA GCA GAT GCC GGC CTT GC-3'
MPO	Forward Primer	5'-CCA GGA AGC CCG GAA GAT-3'
	Reverse Primer	5'-CGG AAG GCA TTG GTG AAG A-3'
	Probe	FAM-TGC CCA CGT ACC GTT CCT ACA ATG ACT C-TAMRA
mMpo	Forward Primer	5'-CCA GGC GGC CCG GAA GAT-3'
	Reverse Primer	5'- TTG GTG AAG ACA TTG GCG AT-3'
RUNX3 3'UTR	Forward Primer	5'-CAG AGA AGA TGA GTC TAT GGC ATC A-3'
	Reverse Primer	5'-GAT GCG TTG AGC TGG TAA AGT G-3'
RUNX3 ex4/5	Forward Primer	5'-CAG CAC CAC AAG CCA CTT CA-3'
(U937/P44)	Reverse Primer	5'-GGT CGG AGA ATG GGT TCA GTT-3'
JMJD3	Forward Primer	5'-GTG ACC TTA GAG AGA GCA GAG TTC AG-3'
	Reverse Primer	5'-CAC GCA GGC GGT GGT T-3'
Cox 5B	Forward Primer	5'-TGG CAT CTG GAG GTG TT-3'
	Reverse Primer	5'-GTC CAG TCC CTT CTT TGC AGC-3'
	Probe	FAM-TGA TGA AGA GCA GGC GAC TGG GTT G-MGB

Supplementary Table 4.

ChIP Primers (Integrated DNA Technologies, INC. Coralville, IA, USA)

hPR3 5'enh	Forward Primer	5'-TGA GAT GGA GTC TTG CTC TGT TG-3'
	Reverse Primer	5'-GGT TGC AGG GAG CCA AGA T-3'
hPR3 prm	Forward Primer	5'-CCA AGG CAA AAG GAG GAA GTG-3'
	Reverse Primer	5'-CAA GCT CCT CTT ATA GCC CAA TG-3'
hPR3 ex3	Forward Primer	5'-CCC ACC CAG CAG CAC TTC-3'
	Reverse Primer	5'-CCG CGT CGT AGT TGT TCA GA-3'
hPR3 in4	Forward Primer	5'-CCC CCA CTC CCC TCT AGG A-3'
	Reverse Primer	5'-CTC CGT CTC AAA AAC AAA CAA AAA G-3'
hPR3 3'UTR	Forward Primer	5'-TCT CGG CTC ACT GCA ACC T-3'
	Reverse Primer	5'-GGA GAC TGA GGC AGG AGA ATT G-3'
hMPO 5'enh	Forward Primer	5'-GGG ACT GGC GGG TAA CTC A-3'
	Reverse Primer	5'-CAA GGG AGT GGG AGG GAT TT-3'
MPO prm	Forward Primer	5'-CAC CCC CAG CTT AGA GGA CAT-3'
	Reverse Primer	5'-TCA CCT GAT ATT GTC AGC TCC TCT T-3'
hMPO in3	Forward Primer	5'-AGA CCT GCT GGA GAG GAA GCT
	Reverse Primer	5'-GCA ACA GTA CCA GTG ACA TTG AAT G-3'
MPO in7	Forward Primer	5'-TCT GGA AGA CAA GAG AGT CAA ATC C-3'
	Reverse Primer	5'-CAC CGC TAG AAA CTG GGT TTA TTT A-3'
hMPO 3'UTR	Forward Primer	5'-CCT CAC CCT GAT TTC TTG CTT ATT-3'
	Reverse Primer	5'-GGG CAA CAG GCC CAG TT-3'
MYO-D	Forward Primer	5'-CCG CCT GAG CAA AGT AAA TGA-3'
	Reverse Primer	5'-GGC AAC CGC TGG TTT GG-3'

RNA polymerase II Immunoprecipitation Primers

PR3	Forward Primer 336	5'-GGT GTT TCT GAA CAA CTA CGA C-3'
	Reverse Primer 624	5'-GAA GCA GAT GCC GGC CTT GC-3'
MPO	Forward Primer	5'-CCA GGA AGC CCG GAA GAT-3'
	Reverse Primer	5'-CGG AAG GCA TTG GTG AAG A-3'
	Probe	FAM-TGC CCA CGT ACC GTT CCT ACA ATG ACT C-TAMRA
Cox 5B	Forward Primer	5'-TGG CAT CTG GAG GTG TT-3'
	Reverse Primer	5'-GTC CAG TCC CTT CTT TGC AGC-3'
	Probe	FAM-TGA TGA AGA GCA GGC GAC TGG GTT G-MGB

Methyl specific PCR Primers

PR3	Methyl Forward Primer	5'-GAT TTT AGC GTT TGG TGA AC-3'
	Unmodified Forward Target	5'-GAC CCC AGC GCC TGG TGA AC-3'
	Methyl Reverse Primer	5'-AAA AAC GTC GTT CAA TTT ATT CT-3'
	Unmodified Reverse Target	5'-GAG AAC GTC GTT CAG TTT GTT CT-3'
	Unmethyl Forward Primer	5'-GTA GAT TTT AGT GTT TGG TGA AT-3'
	Unmodified Forward Target	5'-GTA GAC CCC AGC GCC TGG TGA AC-3'
	Unmethyl Reverse Primer	5'-AAA AAA CAT CAT TCA ATT TAT TCT CC-3'
	Unmodified Reverse Target	5'-GGA GAA CGT CGT TCA GTT TGT TCT CC-3'