Supplementary Methods

Antibody array development

Antibody microarrays were generated using commercially available antibody pairs and standard proteins (R&D Systems, Minneapolis, MN) as previously described [9] using EpoxySilane microarray slides (Erie Scientific, Portsmouth, NH), a Piezoarray™ printer (PerkinElmer, Boston, MA), and the 16 sub-array ProPlate™ system (Grace Bio-labs, Bend, OR). From a panel of 108 proteins that we have developed for assay using this system, 28 were selected on the basis of known or plausible association with AAV and with the goal of addressing diverse processes.

An optimal detection range of each standard curve was developed by testing a dilution series of antigen (0.49-10,000 pg/mL) and a range of secondary antibodies (25-500 ng/mL) that would produce curves with r^2 of 0.95 or greater for quantification. A functioning antibody pair was defined as providing a median fluorescent intensity (MFI) that responded in a dose-dependent manner and only saturated the MFI signal at the higher concentrations. Percent recovery of standards spiked into normal serum was determined.

Controls for specificity (reaction of antigens to multiple capture antibodies, or antibodies to human serum components, or secondary antibodies to capture antibodies) were performed as described [9]. No antigen was recognized by more than one spotted primary antibody. Because several of the tested secondary antibodies did bind nonspecifically to capture antibodies, 3 unique arrays were developed to measure all 28 proteins. As a control for non-specific binding associated with individual samples, isotype controls and non-specific human IgG antibodies were spotted into each sub-array.

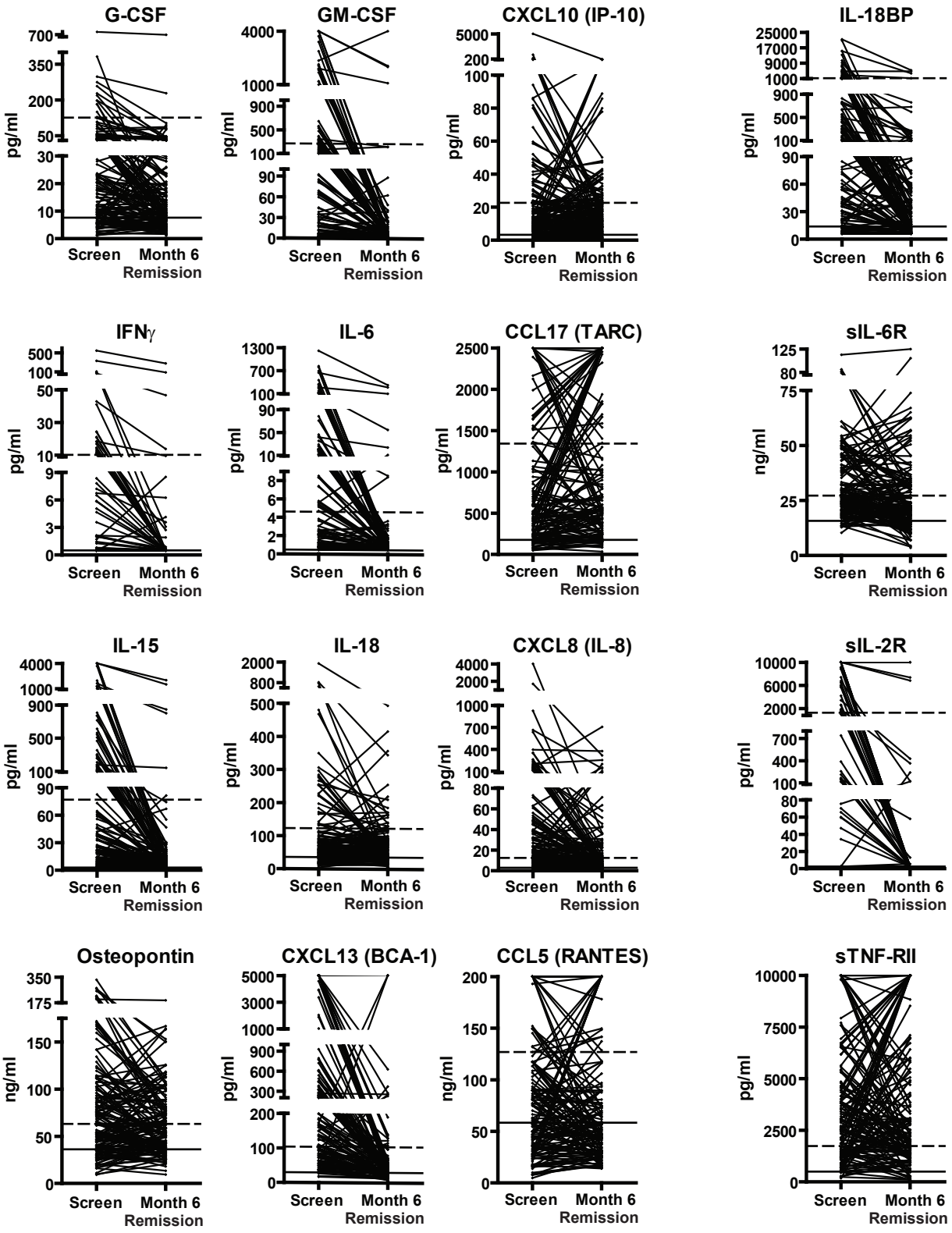
Hybridization and Quantification

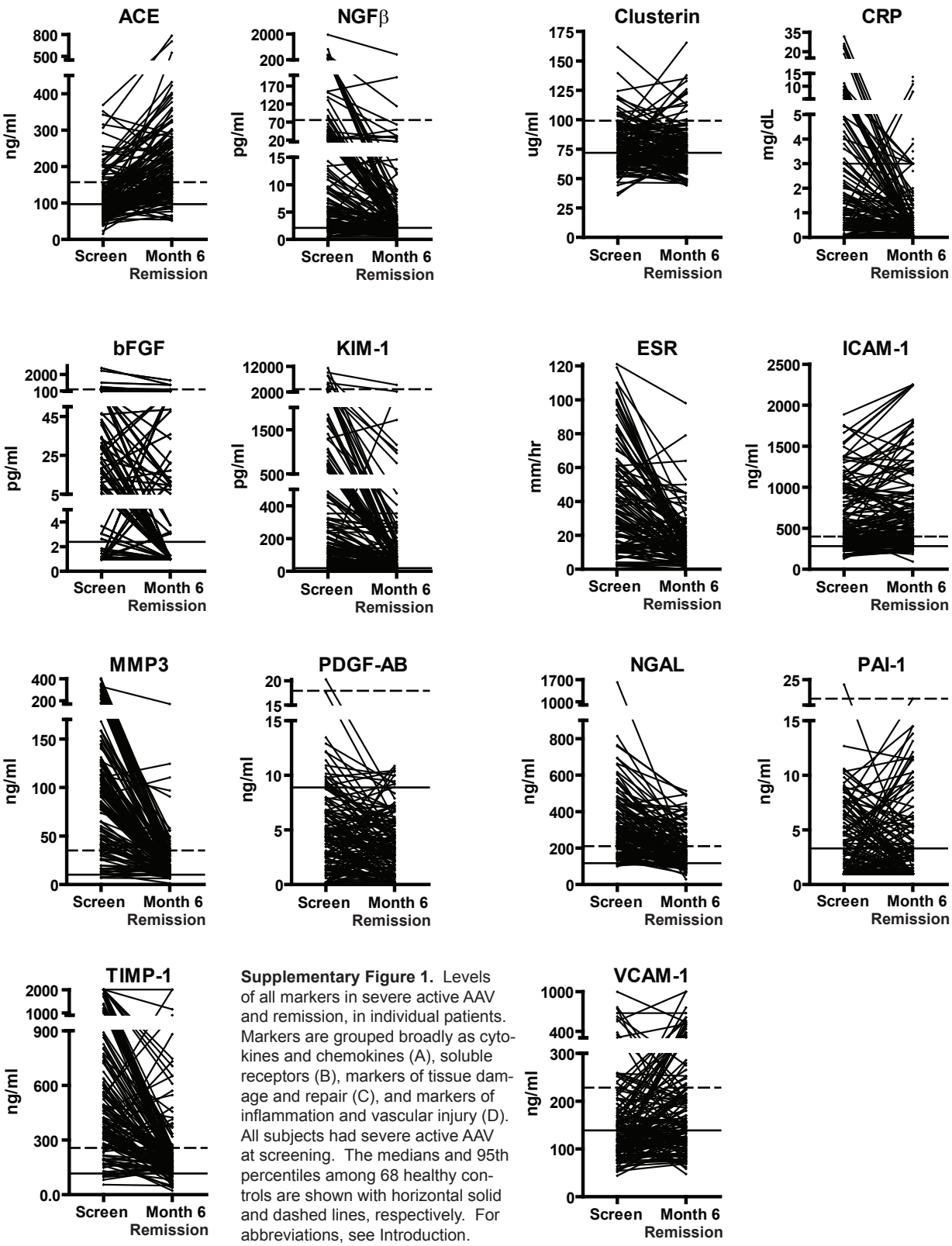
Standards and sera were diluted (5-fold for sera) before hybridization to the arrays as described [9]. Slides were scanned on an Axon 4000B using GenePix 6.0 (Axon Instruments/Molecular Devices, Sunnyvale, CA). A single laser was used and laser intensity was adjusted so the highest standards were not saturated, and the same intensity was used for all slides processed during the same experiment. ProMAT software was used to determine concentrations in samples based on standard curves. The lowest limit of detection for each protein was defined as the first point in the standard curve that showed significantly more signal than the zero point.

Supplementary Table. Marker levels in mildly active AAV and remission at month 6.

Marker*	Month 6 Remission Median (25%,75%) (n=137)	Month 6 Active Median (25%,75%) (n=25)	P	AUC	Sensitivity for Active AAV at Month 6‡
Cytokines					
G-CSF	10.5 (5.63,23.7)	26.4 (13.3,56.0)	0.0014†	0.70	60%
GM-CSF	1.17 (<0.98,4.99)	2.56 (<0.98,25.2)	0.21	0.58	28%
IFNγ	<0.49 (<0.49,<0.49)	<0.49 (<0.49,0.88)	0.018	0.58	
IL-6	<0.49 (<0.49,0.77)	<0.49 (<0.49,2.68)	0.21	0.53	44%
IL-15	5.69 (2.60,13.5)	18.6 (6.69,31.1)	0.0017†	0.62	52%
IL-18	51.9 (31.0,85.8)	48.7 (36.7,102)	0.75	0.52	
Osteopontin	54.4 (37.8,80.9)	46.8 (33.2,74.8)	0.31	0.56	
Chemokines					
BCA-1	32.0 (18.2,55.6)	58.5 (35.9,139)	0.0005†	0.56	44%
IL-8	7.09 (3.59,15.3)	16.4 (4.95,25.1)	0.039	0.45	
IP-10	13.2 (7.68,25.0)	9.91 (5.19,20.1)	0.17	0.59	
RANTES	52.3 (30.8,90.0)	89.5 (39.7,>200)	0.021	0.65	
TARC	655 (347,>2500)	1165 (323,2396)	0.82	0.51	
Soluble Receptors					
IL-18BP	14.6 (<6.11,55.1)	23.6 (8.31,115)	0.13	0.59	32%
sIL-2Ra	<2.44 (<2.44,<2.44)	<2.44 (<2.44,18.2)	0.012	0.59	
sIL-6R	21.9 (15.4,33.0)	23.7 (18.9,34.2)	0.35	0.56	
sTNF-RII	2417 (1350,5808)	2227 (1213,5464)	0.43	0.56	
Tissue Damage and Repair					
ACE	178 (130,252)	146 (119,182)	0.051	0.62	
bFGF	<0.98 (<0.98,9.77)	<0.98 (<0.98,23.9)	0.12	0.57	
KIM-1	45.6 (17.2,127)	63.1 (26.5,274)	0.15	0.58	40%
MMP3	15.6 (11.8,29.1)	27.8 (11.7,64.0)	0.079	0.61	36%
NGFβ	2.48 (1.25,4.32)	2.97 (1.69,14.0)	0.13	0.61	36%
PDGF-AB	3260 (879,5374)	3764 (1558,6180)	0.51	0.54	
TIMP-1	166 (125,233)	303 (157,503)	0.0053†	0.68	56%
Inflammation and Vascular Injury					
Clusterin	73.0 (59.4,85.9)	70.6 (62.7,92.3)	0.82	0.51	
CRP	0.5 (0.3,1.2)	0.65 (0.3,1.25)	0.62	0.55	40%
ESR	14 (7,22)	23 (10,44)	0.021	0.65	44%
ICAM-1	537 (345,882)	682 (461,1003)	0.29	0.57	
NGAL	172 (129,237)	188 (87.5,286)	0.89	0.49	40%
PAI-1	1202 (<977,4719)	1457 (<977,5983)	0.84	0.52	
VCAM-1	148 (108,224)	134 (112,212)	0.79	0.52	

* For abbreviations, see Introduction. BCA-1 = CXCL13; IL-8 = CXCL8; IP-10 = CXCL10; RANTES = CCL5; TARC = CCL17. † Still significant at P<0.05 by Wilcoxon test, after adjustment for multiple comparisons. ‡ Using cut-point established by comparing severe active AAV to remission (see Table 3 in the main text for cut-point values and specificity). For units, see Table 1 in the main text.


A. Cytokines and Chemokines
B. Soluble Receptors
G-CSF
GM-CSF
CXCL10 (IP-10)
IL-18BP
IFNγ
IL-6
CCL17 (TARC)
sIL-6R
IL-15
IL-18
CXCL8 (IL-8)
sIL-2R
Osteopontin
CXCL13 (BCA-1)
CCL5 (RANTES)
sTNF-RII
pg/ml
ng/ml
Screen
Month 6
Remission



Supplementary Figure 1. Levels of all markers in severe active AAV and remission, in individual patients. Markers are grouped broadly as cytokines and chemokines (A), soluble receptors (B), markers of tissue damage and repair (C), and markers of inflammation and vascular injury (D). All subjects had severe active AAV at screening. The medians and 95th percentiles among 68 healthy controls are shown with horizontal solid and dashed lines, respectively. For abbreviations, see Introduction.

APPENDIX

Members of the RAVE-ITN Research Group are as follows: Protocol Co-chairs — U. Specks (Mayo Clinic), J.H. Stone (Massachusetts General Hospital); Mayo Clinic — U. Specks, S.R. Ytterberg, F.C. Fervenza, K.A. Keogh, T. Peikert, J.M. Golbin, L. Klein, K. Mieras, C. Beinhorn, S. Fisher, M.L. Clawson, S. Bendel, A.M. Hummel (Mayo Clinic Eisenberg Research Pharmacy); Boston University — P.A. Merkel, E.Y. Kissin, P.A. Monach, M.R. Clark-Cotton, C.A. McAlear, J.L. Pettit, M.B. Sutton, R.L. Widom, G.A. Farina, M.J. DiMarzio, S.P. Johnson, A. Schiller Patel; Johns Hopkins University — P. Seo, J.H. Stone, D. Hellmann, D. Geetha, A. Saleh, P. Wung, L.P. Sejismundo, C. Humphrey, M. Marriott, Y. Goldsborough, A. Pinachos, K. Gauss, L. King; Cleveland Clinic Foundation — C.A. Langford, G.S. Hoffman, R.A. Hajj-Ali, J.J. Carey, E.S. Molloy, C.L. Koening, D. Bork, T.M. Clark, K.A. Tuthill, T. Markle, J. Petrich; Hospital for Special Surgery — R. Spiera, D.R. Alpert, S.J. DiMartino, J.K. Gordon, N.K. Moskowitz, K.A. Kirou, J. Samuels, S.A. Kloiber, E. Julevic, M. O'Donohue, A. Patel; University of Groningen — C.G.M. Kallenberg, C. Stegeman, P. Rasker, K. Mulder, P. Limburg, J. Kosterink; Duke University — E.W. St. Clair, N.B. Allen, E. Scarlett, M. Tochacek; University of Alabama–Birmingham — A. Turkiewicz, B. Fessler, W. Chatham, A. Turner; Coordinating Centers: Rho — D. Ikle, D. Weitzenkamp, W. Wu, T. D'Lugin, C. Jacob; National Institute of Allergy and Infectious Diseases — L. Webber, L. Ding, S. Adah; Immune Tolerance Network — N.K. Tchao, M. Mueller, K. Bourcier, A. Asare, V. Seyfert-Margolis, P. Tosta, N.B. Skeeter, C.L. Anderson, A.N. Archampong.