Supplementary File

Materials and Methods

Study subjects

Aliquots of archived serum samples from patients with class III and/or IV lupus proliferative nephritis enrolled in the LUNAR trial[1], a randomized, double-blinded, placebo-controlled phase III trial of rituximab or placebo infusions (given on days 1, 15, 168 and 182) given concomitantly with MMF and corticosteroids.

Antibodies and antigens

Patient TII mAbs were generated as previously published [2]. Commercial murine mAbs reactive with the C-terminus of Vimentin (clone V9 (DAKO, Glostrup, Denmark) and E5 (Santa-Cruz, Santa-Cruz, CA, USA) and cytokeratin 18 (clone DC10 (DAKO, Glostrup, Denmark)). Mammalian vimentin purified from bovine eye lens was from SIGMA-ALDRICH (St Louis, MO, USA).

Vimentin cloning and protein purification

Vimentin was cloned into the expression vector pET-24b (to be expressed as full-length vimentin$_{1\text{-}466}$ C-terminal 6 His-tag fusion) by NMV *et al*. An analogous recombinant C-terminal vimentin$_{259\text{-}466}$ construct was generated by AK as previously outlined[3]**,** based on the construct developed by Cha.et.al[4]. Expression and purification was performed by AK [3]. Briefly, expression was induced in BL21 Rosetta (DE3) pLysS (EMD, Madison, WI, USA) cells, cultures pelleted and inclusion bodies solubilised in 8M urea 50mM Tris-HCl, pH 8.0 10mM

beta-mercaptoethanol. Vimentin was recovered from the supernatant by incubating with Ni-agarose resin overnight at 4°C and then stepwise washing with decreasing acidity from pH8.0 to pH3.2. Eluates containing pure (as determined by Coomassie staining) vimentin were pooled and dialysed against 50mM Tris-HCl pH8.0, 10mM beta-mercaptoethanol, 5% glycerol, concentrated by size exclusion chromatography, aliquoted and snap frozen for storage in liquid nitrogen at 1.0mg/ml. *In vitro* citrullination was performed as described previously[5].

ELISAs

Anti-vimentin antibody ELISAs were performed using thawed vimentin aliquots, firstly diluted to 200μg/ml in 8M Urea pH8.0, 10mM beta-mercaptoethanol, and then to 10 μg/ml in PBS before coating wells of ELISA plates (Costar 96-well clear, flat-bottom, half-area high-binding polystyrene plates, Corning cat.no 3690, New York, USA). Control wells, coated with bovine vimentin or BSA (diluted as for recombinant human vimentin) were added where indicated. Antigens were attached overnight at 4°C. Plates were rinsed of unattached antigen with ddH_20 and blocked in blocking buffer (PBS/3% BSA) for 3 hours. Monoclonal antibodies were added at the concentrations indicated for 1.5 hours, washed 3 times in .01% tween and detected with HRP conjugated secondary antibodies (anti-human IgG (Jackson ImmunoResearch, West Grove, PA, USA (109-035-098)) or anti-mouse IgG (GE Healthcare UK, (NXA931V)) diluted 1:1000 in blocking buffer. Following washing (as above), Super AquaBlue (eBioscience, San Diego, CA, USA) was added and optical density measured at 405 ηm. Serum (diluted 1/50 in blocking buffer) was incubated in the well for 1.5 hours and washed five times with PBS/Tween 0.1%. Bound IgG, IgA and IgM were separately detected using HRP-conjugated secondary antibodies (109-035-098, 109-035-011 or 109-035-129, Jackson ImmunoResearch, West Grove, PA, USA)

diluted 1:1000 in blocking buffer. Following washing (as for the primary incubation), Super AquaBlue (eBioscience, San Diego, CA, USA) was added and optical density measured at 405 ηm. For each serum AVA isotype titration, a standard (frozen aliquots of pooled AVA^{hi} lupus sera (assigned a value of 1000 arbritrary units [AU])) was added at serial two-fold dilutions (in duplicate). Using the standard curve, sample titres were interpolated and expressed as AVA AU. Commercially available clinical ELISAs performed as per manufacturers' instructions on the TRIDOM cohort were (Inova Diagnostics) IgG anti-dsDNA (QUANTA Lite 708510), IgG anti-RNP (QUANTA Lite 708565), IgG anti-Sm (QUANTA Lite 708560), IgG anti-CCP3 (QUANTA Lite 704535), and RF (IgM (QUANTA Lite 708690), IgG (QUANTA Lite 708685) and IgA (QUANTA Lite 708695)). Non-AVA antibody titres, with the exception of IgG anti-β_2GPI (QUANTA Lite 708665) in the LUNAR cohort were acquired during and subsequent to the original 52 week multi-center study [1]. Double stranded DNA, SSA, SSB, Sm, RNP were measured with the AtheNA MultiLyte ANA Test System (Zeus Scientific). ANA were measured using HEp-2 indirect immunofluorescence (Kallestad ANA kit (Bio-Rad)) and anti-cardiolipin antibody (ACA) titres were measured by ELISA (QUANTA Lite 708625 ACA IgG III).

Statistical analysis

Longitudinal analyses (**Figure 4** and **Figure S5**) of the effects of AVA, and IgG anti-$Vim_{C\text{-}term}$, titre data were merged into the previously curated LUNAR clinical dataset. A linear mixed effect model with saturated design was fit to capture the longitudinal association. Patient level random intercepts and slopes in time were taken into account for intrasubject temporal correlation. In this exploratory analysis, UPCR, serum creatinine, soluble complement factor C3, and anti-dsDNA were considered as response variables respectively. The longitudinal association was specified to

be the linear relationship between the change in the response variable from baseline to visit and the AVA status (adjusting for the response variable value at baseline). The temporal trends were plotted using the fitted mean marginal responses per visit for the respective AVA status. The contrast between the two groups for each time point was calculated. Standard errors of marginal response and contrast were estimated using the delta method. The standardised contrast was used to infer the significance of the difference between AVA status subgroup for each visit. The effect of each term in the mixed effect model was estimated using ANOVA on the fitted model.

Tables

Table S1. Histological Classifications of Respective Subjects with Nephritis in the TRIDOM Mixed Lupus Cross-Sectional Cohort			
ISN/RPS Lupus Nephritis Class(es)	**NIH Activity Index**	**NIH Chronicity Index**	**TII**
Proliferative			
III	7	0	1
III	8	0	1
III	9	0	1
III	0	1	1
III	8	2	3
III	6	2	1
III, V	5	6	3
III	11	4	3
III, V	5	1	0
III, V	4	1	0
III, V	3	1	2
III, V	3	3	1
III, V	7	2	1
IV	2	9	2
IV	19	4	3
IV, V	16	2	3
IV, V	4	3	3
Non-Proliferative			
II, V	1	3	2
V	2	3	2
V	0	1	0
V	#	#	0
V	#	#	1
ISN/RPS = International Society of Nephrology/ Renal Pathology Society; NIH = National Institutes of Health; TII = Tubulointerstitial Inflammation # = Data Not Available			

Table S2i. Correlations of AVA Titres with Lupus and RA associated Autoantibodies in the TRIDOM Mixed Lupus Cohort (n=99)				
AVA Isotype	**Correlating Autoantibody**	**Spearman r**	**95%CI**	***p***
IgG AVA	IgG AVA	NA	NA	NA
	IgA AVA	0.329	0.131 to 0.502	0.0011**
	IgM AVA	0.095	-0.114 to 0.297	0.3577
	IgG anti-RNP	-0.195	-0.388 to 0.014	0.0593
	IgG anti-Sm	-0.250	-0.438 to -0.042	0.0161*
	IgG anti-dsDNA	-0.102	-0.303 to 0.108	0.3268
	IgG RF	-0.022	-0.23 to 0.187	0.8321
	IgM RF	0.174	-0.040 to 0.372	0.0994
	IgA RF	0.018	-0.192 to 0.227	0.8652
	IgG anti-CCP3	0.274	0.067 to 0.458	0.0082**
IgA AVA	IgG AVA	0.329	0.131 to 0.502	0.0011**
	IgA AVA	NA	NA	NA
	IgM AVA	0.184	-0.022 to 0.375	0.0718
	IgG anti-RNP	0.055	-0.153 to 0.258	0.5942
	IgG anti-Sm	0.057	-0.153 to 0.263	0.5825
	IgG anti-dsDNA	-0.054	-0.256 to 0.153	0.6015
	IgG RF	0.089	-0.119 to 0.29	0.3866
	IgM RF	0.283	0.077 to 0.466	0.0063**
	IgA RF	0.293	0.0908 to 0.471	0.0040**
	IgG anti-CCP3	0.335	0.136 to 0.508	0.0010***
IgM AVA	IgG AVA	0.095	-0.114 to 0.297	0.3577
	IgA AVA	0.184	-0.022 to 0.375	0.0718
	IgM AVA	NA	NA	NA
	IgG anti-RNP	-0.036	-0.240 to 0.170	0.7250
	IgG anti-Sm	0.009	-0.199 to 0.216	0.9311
	IgG anti-dsDNA	0.130	-0.078 to 0.326	0.2056
	IgG RF	0.450	.269 to 0.601	<0.0001****
	IgM RF	0.560	0.397 to 0.688	<0.0001****
	IgA RF	0.029	-0.178 to 0.234	0.7794
	IgG anti-CCP3	0.218	0.011 to 0.407	0.0337*

CCP3=cyclic citrullinated peptide 3; ANA=anti-nuclear antibody; AVA=Anti-vimentin antibodies; dsDNA=double stranded DNA; 95% CI=95% confidence interval; p=probability; NA=not applicable; RNP=ribonuclear protein; Sm=Smith; RF=rheumatoid factor; SSB=Sjögren's syndrome antigen B; $Vim_{C\text{-}term}$=vimentin C-terminus. Color code: light grey: lupus associated antibodies; dark grey: RA associated antibodies.

Table S2ii. Association of Autoantibody Titres with Different Disease Manifestations in the TRIDOM cohort

Manifestion Frequency	**Muco** 22(77)	**Card & Resp** 2(97)	**Immunol** 55(44)	**Hematol** 15(84)	**Neph** 22(77)	**Musc** 9(90)
AutoAntibody						
IgG dsDNA	261(275)	204(274)	410(125)****	248(274)	318(242)	138(274)
IgM AVA	121(118)	86(118)	139(110)	138(117)	118(116)	143(117)
IgG AVA	114(115)	205(115)	104(121)	99(116)	43(122)*	162(104)*
IgA AVA	105(114)	526(110)	97(121)	147(107)	85(123)	223(105)****
IgM RF	7(6)	7(6)	6(6)	7(6)	5(6)	8(6)*
IgG CCP	8(8)	7(8)	8(8)	9(8)	7(8)	12(8)
IgG RF	5(5)	6(5)	5(5)	5(5)	5(5)	7(5)*
IgA RF	5(5)	6(5)	5(5)	5(5)	5(5)	8(5)*
IgG Sm	27(22)	59(21)	28(14)**	42(21)**	32(19)*	9(24)
IgG RNP	57(62)	117(59)	93(30)**	126(45)	49(71)	11(64)

*Frequencies of patients, with (without), different disease manifestations, and median autoantibody titres. Comparisons between respective antibody titres of patients with and without indicated manifestations were made using the Mann-Whitney U test. Statistically different titres are indicated (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001). Card & Resp=cardiological & respiratory; Hematol=hematological; Immunol=immunological; Muco=mucocutaneous; Neph=nephritic. Other manifestations, vascular (n=1) and neuropsychiatric (n=0) were not plotted due to low incidence.*

Table S2iii. Association of Autoantibody Titres with Different Degrees of TII in TRIDOM LN patients (n=22)

TII score frequency	**0** (n=4)	**1** (n=8)	**2** (n=4)	**3** (n=6)
Autoantibody				
IgG dsDNA	318	250	303	386
IgM AVA	130	130	107	101
IgG AVA	30	41	83	103
IgA AVA	96	99	58	94
IgM RF	7	6	3	5
IgG CCP	5	8	6	10
IgG RF	5	5	5	5
IgA RF	5	5	5	4
IgG Sm	32	35	18	68
IgG RNP	131	35	13	59

Frequencies of LN patients with different TII scores and median titres of indicated autoantibodies. Insufficiently low frequencies in respective TII groups were not sufficiently powerful for robust statistical analyses.

Table S3. Correlations of Baseline AVA titres with Other Autoantibodies in the LUNAR Proliferative Lupus Nephritis Cohort (n=131)				
AVA Isotype	**Correlating Autoantibody**	**Spearman r**	**95%CI**	***p***
IgG AVA	IgG AVA	NA	NA	NA
	IgG $Vim_{C\text{-}term}$	0.533	0.394 to 0.648	<0.0001****
	IgA AVA	0.487	0.340 to 0.610	<0.0001****
	IgM AVA	0.082	-0.0953 to 0.254	0.3507
	IgG anti-RNP	0.136	-0.0409 to 0.304	0.1203
	IgG anti-Sm	0.100	-0.077 to 0.271	0.2531
	IgG anti-dsDNA	0.245	0.0719 to 0.403	0.0047**
	IgG ACA	0.299	0.130 to 0.451	0.0005***
	IgG anti-SSA	0.056	-0.121 to 0.229	0.5263
	IgG anti-SSB	0.172	-0.00358 to 0.338	0.0481*
	IgG ANA	0.149	-0.0285 to 0.317	0.0901
	IgG β2GPI	0.156	-0.0220 to 0.324	0.0768
IgA AVA	IgG AVA	0.487	0.34 to 0.61	<0.0001****
	IgG $Vim_{C\text{-}term}$	0.410	0.252 to 0.546	<0.0001****
	IgA AVA	NA	NA	NA
	IgM AVA	0.176	-7.16e-005 to 0.341	0.0438*
	IgG anti-RNP	0.202	0.0272 to 0.365	0.0201*
	IgG anti-Sm	0.220	0.0457 to 0.381	0.0114*
	IgG anti-dsDNA	0.173	-0.00257 to 0.339	0.0469*
	IgG ACA	0.195	0.0195 to 0.358	0.0253*
	IgG anti-SSA	0.134	-0.043 to 0.303	0.1261
	IgG anti-SSB	0.214	0.0395 to 0.375	0.0138*
	IgG ANA	0.119	-0.0583 to 0.29	0.1743
	IgG β2GPI	0.130	-0.0483 to 0.300	0.1405
IgM AVA	IgG AVA	0.0819	-0.0953 to 0.254	0.3507
	IgG $Vim_{C\text{-}term}$	0.125	-0.0515 to 0.295	0.1517
	IgA AVA	0.176	-7.16e-005 to 0.341	0.0438*
	IgM AVA	NA	NA	NA
	IgG anti-RNP	0.174	-0.00204 to 0.339	0.0462*
	IgG anti-Sm	0.082	-0.0953 to 0.254	0.3505
	IgG anti-dsDNA	0.053	-0.124 to 0.227	0.5458
	IgG ACA	0.126	-0.0506 to 0.296	0.1488
	IgG anti-SSA	0.016	-0.16 to 0.191	0.8572
	IgG anti-SSB	0.095	-0.0818 to 0.267	0.2767
	IgG ANA	0.106	-0.0716 to 0.277	0.2273
	IgG β2GPI	0.215	0.0397 to 0.378	0.0139*

ACA=anti-cardiolipin; ANA=anti-nuclear antibody; AVA=Anti-vimentin antibodies; β2GPI=β2 glycoprotein I; dsDNA=double stranded DNA; RNP=ribonuclear protein; Sm=Smith; SSA=Sjogren's syndrome antigen A; SSB=Sjögren's syndrome antigen B; $Vim_{C\text{-}term}$=vimentin C-terminus residues 259-466. 95% CI=95% confidence interval; p=probability; NA=not applicable.

Table S4 Baseline Characteristics of AVA High and Low Groups									
	IgG AVA			IgA AVA			IgM AVA		
	High	***Low***	***p***	***High***	***Low***	***p***	***High***	***Low***	***p***
UPCR	3.4 ± 2.8	4.4 ± 2.6	0.018*	3.4 ± 3.1	4.5 ± 2.2	0.036*	4.4 ± 3.2	4.2 ± 2.6	0.925
SCr	1.0 ± 0.5	1.0 ± 0.5	0.80	1.0 ± 0.6	1.0 ± 0.4	0.30	1.0 ±0.5	1.1 ± 0.5	0.066
C3	69 ± 27	76 ± 28	0.17	69 ±76	76 ± 27	0.13	72.2 ± 31.6	75.2 ± 27.4	0.424
dsDNA	606 ± 432	242 ± 953	0.008**	476 ± 653	370 ± 853	0.20	603.2 ± 849.7	407.5 ± 560.5	0.053
Values are reported as mean ± SD. Comparisons were made using the Mann-Whitney U test; p=probability of difference between two groups.									

Table S5 LUNAR patient change in UPCR at week 78 by AVA baseline status			
	Mean UPCR (g/g) change at week 78 by AVA baseline status		**Difference in UPCR (g/g) change between AVA high and low groups**
AVA subtype	*high*	*low*	
IgG AVA	-1.90	-3.32	1.42
IgA AVA	-2.20	-3.08	0.88
IgG anti-Vim$_{C\text{-}term}$	-2.22	-3.04	0.82
IgM AVA	-2.78	-2.52	-0.26

Table S6 LUNAR patient change in serum creatinine at week 78 by AVA baseline status (for respective treatment groups)			
	Mean change (mg/dL) at week 78 by AVA baseline status		**Difference in change between AVA high and low groups**
i.Rituximab			
AVA subtype	*high*	*low*	
IgG AVA	0.22	-0.18	0.40
IgA AVA	0.13	-0.11	0.24
IgG anti-Vim$_{C\text{-}term}$	0.08	-0.07	0.15
IgM AVA	-0.03	0.05	-0.08
ii.Placebo			
AVA subtype	*high*	*low*	
IgG AVA	-0.10	-0.10	0.00
IgA AVA	-0.11	-0.09	-0.02
IgG anti-Vim$_{C\text{-}term}$	-0.08	-0.12	0.04
IgM AVA	-0.18	-0.04	-0.14

Figures

Figure S1

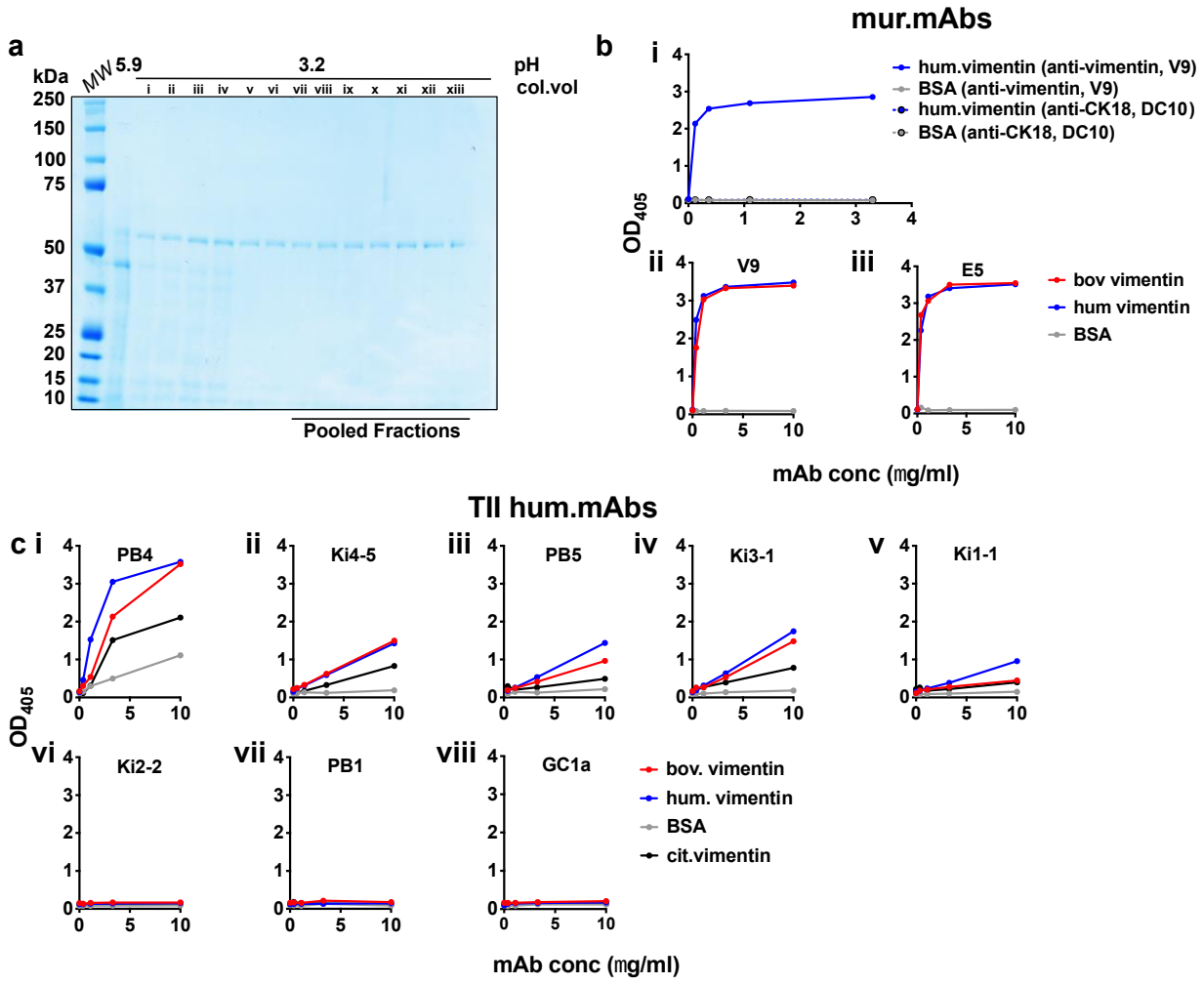

Figure S1 *Confirmation of AVA reactivity with unmodified recombinant human vimentin.* (**a**) Coomassie stain of SDS-PAGE resolved recombinant His-tagged full-length vimentin$_{1\text{-}466}$ fractions eluted from Ni-NTA agarose beads in consecutive column volumes (col.vols) of 8M urea adjusted to indicated pH (col.vol, 5µl sample of each col.vol. was loaded per lane). Eluates without visible contaminant bands were pooled for ELISA (**b**) Reactivity of commercially available murine anti-vimentin (clone V9) and anti-cytokeratin 18 (-CK18, clone DC10) mAbs with recombinant human (hum.) vimentin and BSA coated wells by ELISA (**i**), and murine anti-

vimentin clones V9 (**ii**) and E5 (**iii**) with bovine eye lens antigen, recombinant human antigen and BSA. (**c**) Reactivity of antigens with different human TII mAbs cloned from activated B-cells from within the inflamed tubulointerstitium[2]. bov=bovine; hum=human, cit=citrullinated. col.vol.=column volume.

Figure S2

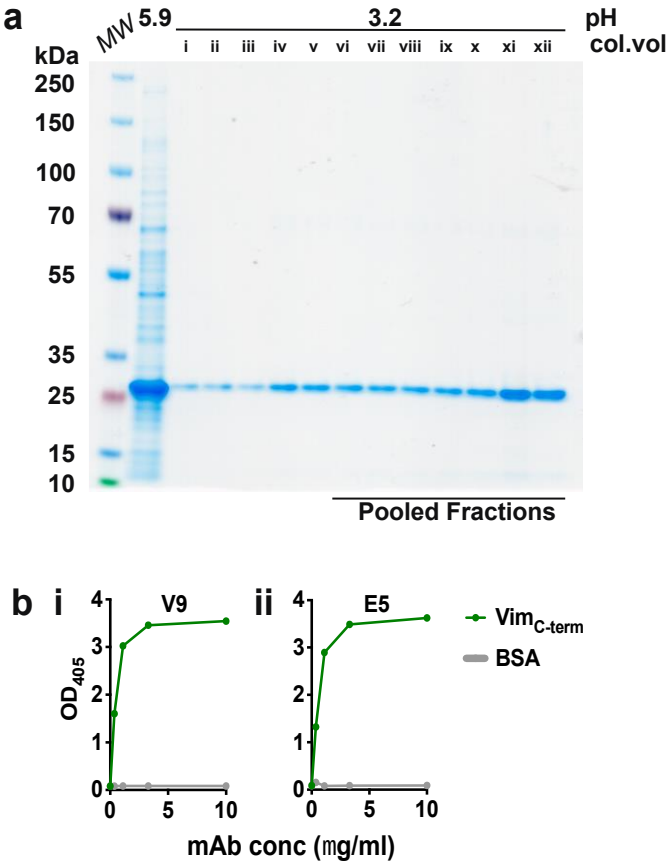

Figure S2 *Confirmation of purity of, and AVA reactivity with, recombinant truncation of human vimentin (amino acids 259—466).* (**a**) Coomassie stain of recombinant His-tagged vimentin$_{260\text{-}467}$ (Vim$_{C\text{-}term}$) fractions eluted from Ni-NTA agarose beads as for **Figure S1a**. (**b**) Reactivity of commercially available murine anti-vimentin (clones V9 (**i**) and E5 (**ii**)) mAbs with Vim$_{C\text{-}term}$ and BSA coated wells by ELISA.

Figure S3

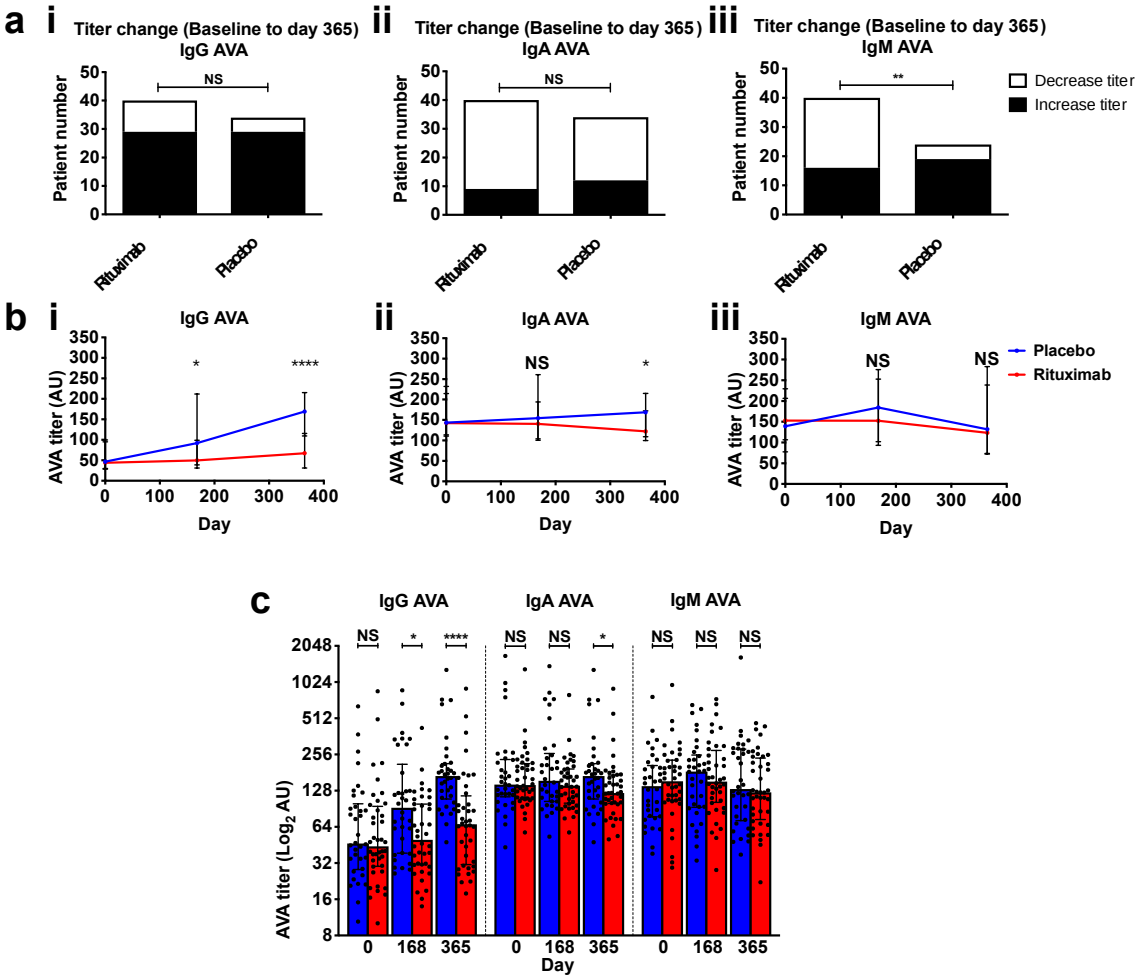

Figure S3 *Effect of different treatments on titres of AVAs in LUNAR patients.* (**a**) Frequency of indicated AVA isotype titre that increased or decreased from baseline to day 365. Comparisons between frequencies were performed the treatment groups using Fisher's exact test. (**b**) Average respective AVA isotype titres at days 0, 168 and 365. Medians and IQRs from each group of patients' titres are plotted for respective treatments. Mann Whitney tests were performed between treatments for each time point. (**c**) Individual patient AVA titres at different time points for respective treatments. Medians and IQRs from each group of patients' titres were plotted for respective treatments and Mann Whitney tests were performed between treatments for each time

point. Each dot represents an individual patient's AVA titre. $0.01<*p<0.05$, $0.01<**p<0.001$, $0.001<***p<0.0001$, $****p<0.0001$.

Figure S4

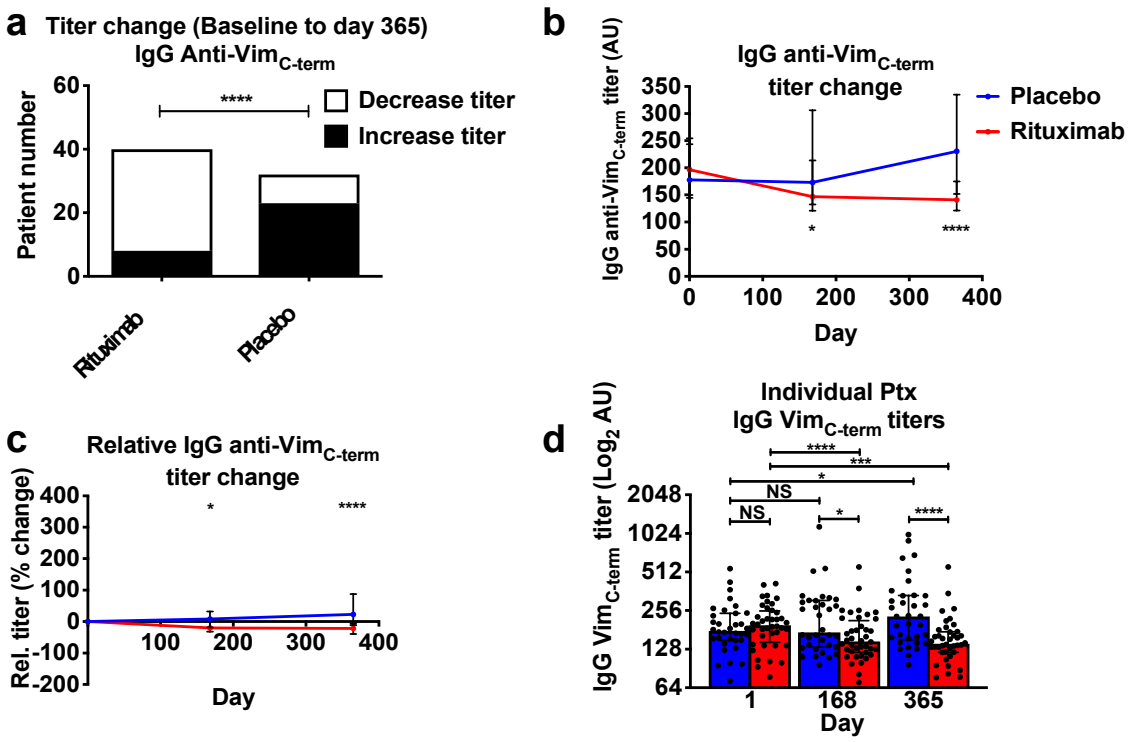

Figure S4 *Effect of different treatment regimens on titres of IgG anti-Vim$_{C\text{-}term}$ as for* **Fig 3** *and* **Figure S3**. (**a**) Frequency of titre increase from baseline to day 365 in the two treatment groups. Comparisons were performed using Fisher's exact test. (**b**) IgG anti-Vim$_{C\text{-}term}$ titres at days 0,168 and 365. (**c**) Relative (percentage) change in IgG anti-Vim$_{C\text{-}term}$ from baseline. (**d**) Raw titres at different time points for respective treatments. Each dot represents an individual patient's IgG anti-Vim$_{C\text{-}term}$ titre. 0.01<*p<0.05, 0.01<**p<0.001, 0.001<***p<0.0001, ****p<0.0001, NS=non-significant.

Figure S5

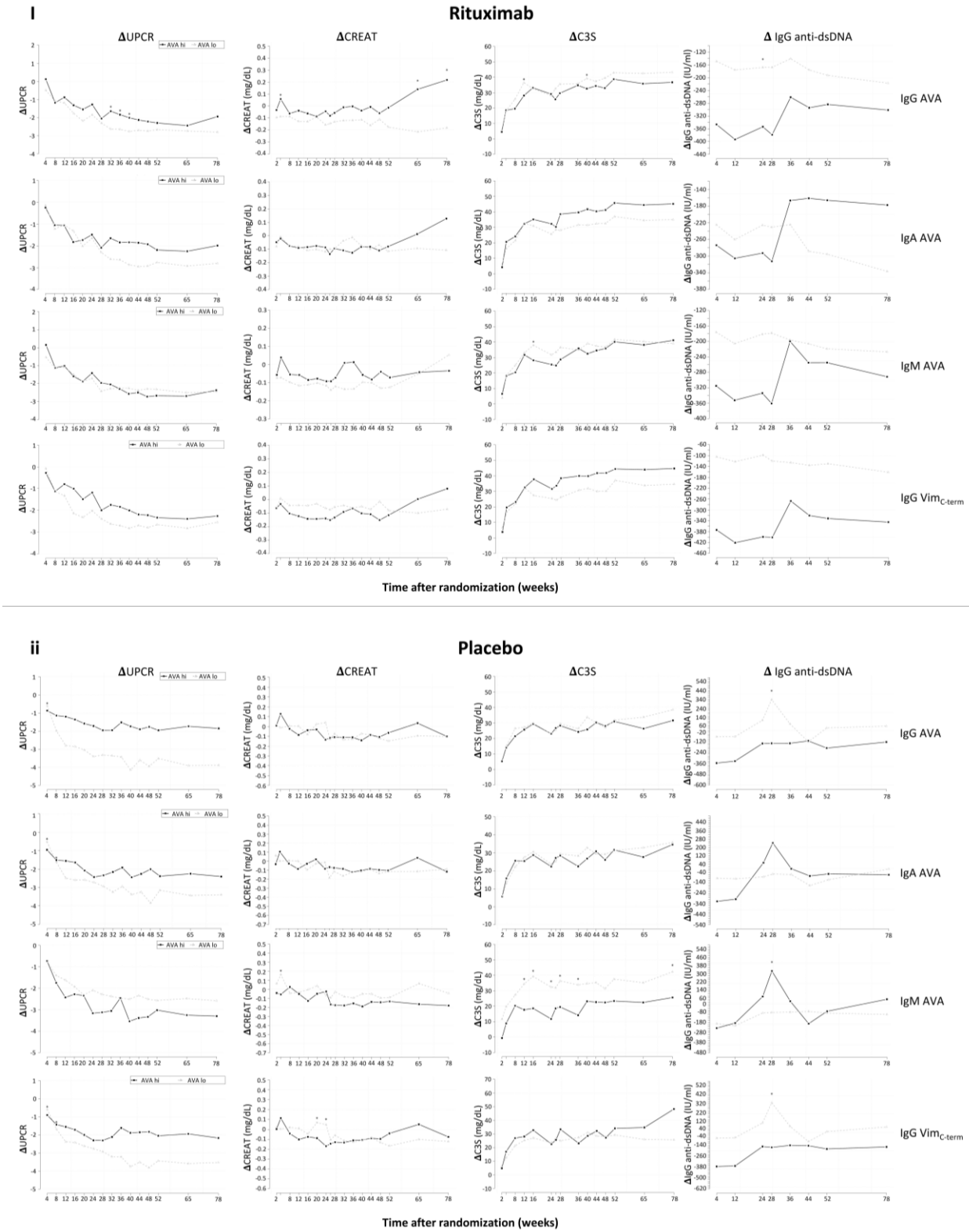
I
Rituximab
ΔUPCR
ΔCREAT
ΔC3S
Δ IgG anti-dsDNA
AVA hi
AVA lo
IgG AVA
IgA AVA
IgM AVA
IgG Vim C-term
Time after randomization (weeks)
ii
Placebo
ΔUPCR
ΔCREAT
ΔC3S
Δ IgG anti-dsDNA
AVA hi
AVA lo
IgG AVA
IgA AVA
IgM AVA
IgG Vim C-term
Time after randomization (weeks)

Figure S5 *Disease Metric changes (***Δ**) *from baseline in patients with high or low AVA titres (by treatment group).* For each AVA isotype, patients on respective treatment regimens were divided into AVA high (above median baseline AVA isotype titre) or AVA low (below median baseline AVA isotype titre) groups. Mann Whitney tests were performed between groups for each time point, and where significant indicated. 0.01<*p<0.01. Rituximab (**i**) and placebo (**ii**) groups were plotted separately.

UPCR=Urine protein/serum creatinine ratio; CREAT=serum creatinine; C3S=soluble complement factor C3.

Supplementary File References

1. Rovin, B.H., et al., *Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study.* Arthritis Rheum, 2012. **64**(4): p. 1215-26.
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3. Kinloch, A.J., et al., *In Situ Humoral Immunity to Vimentin in HLA-DRB1*03(+) Patients with Pulmonary Sarcoidosis.* Front Immunol, 2018. **9**: p. 1516.
4. Cha, S.C., et al., *Nonstereotyped lymphoma B cell receptors recognize vimentin as a shared autoantigen.* J Immunol, 2013. **190**(9): p. 4887-98.
5. Kinloch, A., et al., *Identification of citrullinated alpha-enolase as a candidate autoantigen in rheumatoid arthritis.* Arthritis Res Ther, 2005. **7**(6): p. R1421-9.