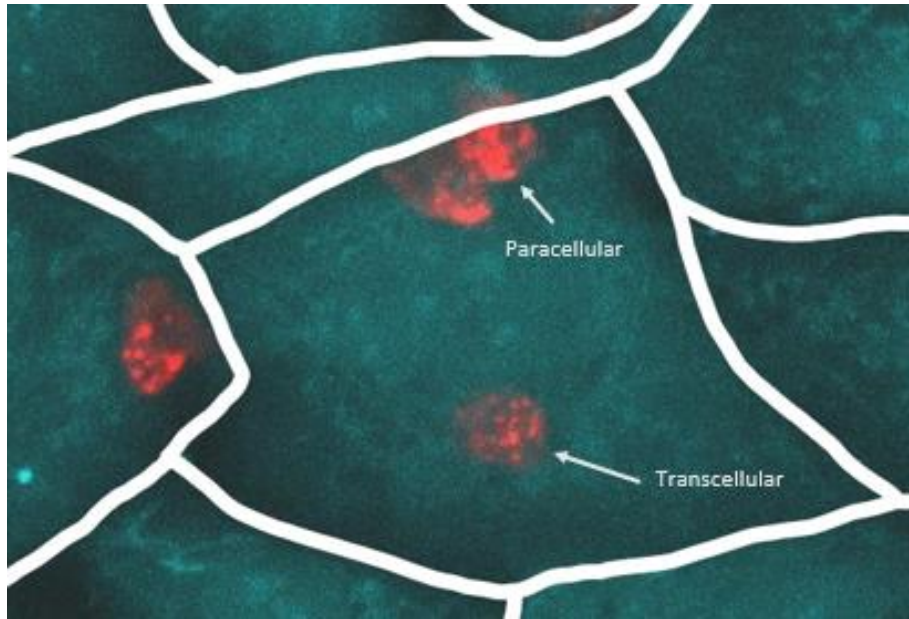


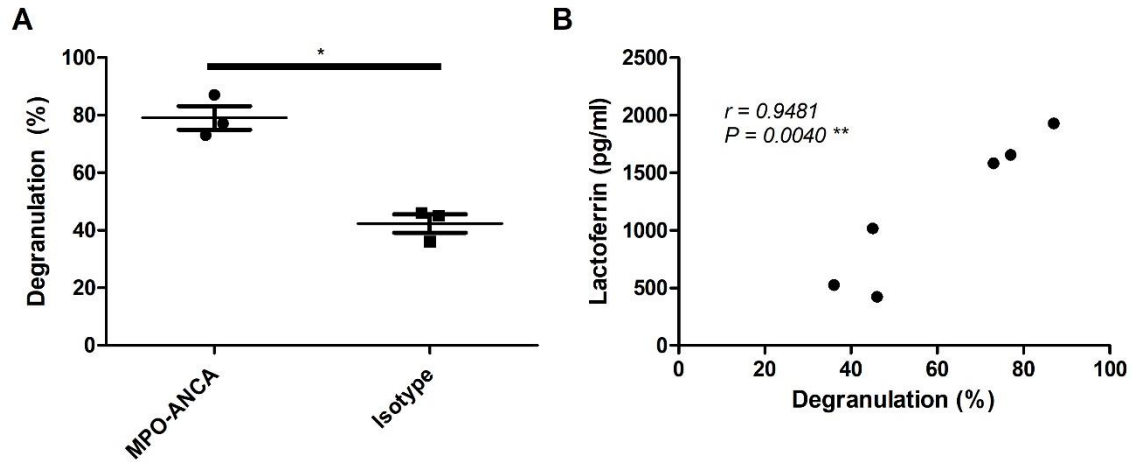
## Supplemental Data

**Table 1** - Patient Characteristics (C5aR1 antagonist experiment)

Variables	
	<i>n</i> = 6
Age, median (range), years	77 (43-88)
Sex, male	2 (33%)
AAV Diagnosis	
○ GPA	1 (17%)
○ MPA	5 (83%)
○ EGPA	0 (0%)
Organ Involvement	
Constitutional	4 (67%)
Renal	5 (83%)
Chest	3 (50%)
ENT	1 (17%)
Nervous	0 (0%)
Cutaneous	0 (0%)
Mucous membranes / eyes	1 (17%)
Disease duration, median (range), months	0.84 (0.24 – 24)
BVAS Score, median (range)	11.5 (6 – 17)
ANCA status at time of sampling	
MPO-ANCA	6 (100%)
PR3-ANCA	0 (0%)
ANCA-negative	0 (0%)
Serum Creatinine, median (range) (μM/L)	185.5 (74 – 1010)
Raised CRP > 4 mg/L	3 (50%)
eGFR < 60 mL/min	5 (83%)
Therapy	
Prednisolone	6 (100%)
Cyclophosphamide	3 (50%)
Rituximab	1 (17%)
Azathioprine	1 (17%)



**Figure 1** - Image depicting protocol for measurement of transcellular or paracellular transmigration. Transmigration of leukocytes in the blue area (i.e. through endothelial cell body) was quantified as transcellular and along the white area (i.e. through the endothelial cell junctions), paracellular.



**Figure 2** – Monoclonal MPO-ANCA stimulation of neutrophil degranulation. A) Healthy donor neutrophils were stimulated with 2ng/ml TNF- $\alpha$  and then incubated with 5ug/ml MPO-ANCA, or isotype control, to induce degranulation in the 4-D imaging system (Data shown is mean  $\pm$  SEM; paired t-test; \*  $P < 0.05$ ;  $n = 3$ ). B) Neutrophil degranulation was then quantified visually and correlated with lactoferrin (Pearson correlation  $r = 0.9481$ ;  $P = 0.0040$ ).

**Table 2** - Leukocyte Dynamics (autologous serum)

Neutrophil (mean $\pm$ SEM)	Active Patient	Remission Patients	Healthy Donor
Degranulation (%)	74.4 $\pm$ 4.8	46.6 $\pm$ 5.8	45.2 $\pm$ 4.5
Track Velocity ( $\mu\text{m}/\text{min}$ )	2.1 $\pm$ 0.1	1.9 $\pm$ 0.2	2.3 $\pm$ 0.2
Transmigration (%)	52.7 $\pm$ 4.6	35.8 $\pm$ 8.4	28.7 $\pm$ 5.8
Transcellular Transmigration (%)	39.1 $\pm$ 6.3	28 $\pm$ 9.1	14.2 $\pm$ 11.0
Monocyte (mean $\pm$ SEM)			
Degranulation (%)	31.0 $\pm$ 3.7	29 $\pm$ 5.6	21.9 $\pm$ 2.9
Track Velocity ( $\mu\text{m}/\text{min}$ )	1.8 $\pm$ 0.4	1.6 $\pm$ 0.3	1.9 $\pm$ 0.3
Transmigration (%)	67.9 $\pm$ 2.5	59.7 $\pm$ 9.3	57.5 $\pm$ 7.0
Transcellular Transmigration (%)	47.6 $\pm$ 4.0	42 $\pm$ 5.9	20.7 $\pm$ 6.0

**Table 3 – Leukocyte Dynamics (C5aR1 antagonism)**

Neutrophil (mean ± SEM)	C5aR1	DMSO	<i>p-value</i>
Degranulation (%)	35.7 ± 4.1	53.8 ± 4.2	0.0002
Track Velocity (µm/min)	0.89 ± 0.27	0.78 ± 0.19	0.3586
Transmigration (%)	60.8 ± 9.6	58.6 ± 7.4	0.8716
Transmigration total distance (µm)	2.3 ± 0.5	2.5 ± 0.6	0.8308
Transcellular Transmigration (%)	36.9 ± 8.7	40.9 ± 13.1	0.7813
<b>Monocyte (mean ± SEM)</b>			
Degranulation (%)	21.0 ± 10.3	20.7 ± 7.5	0.9530
Track Velocity (µm/min)	0.57 ± 0.13	0.74 ± 0.15	0.0440
Transmigration (%)	73.0 ± 3.9	72.8 ± 8.19	0.9847
Transmigration total distance (µm)	2.8 ± 0.16	3.9 ± 1.12	0.3480
Transcellular Transmigration (%)	35.3 ± 8.8	27.5 ± 5.5	0.3845

**Table 4 – Leukocyte Dynamics (polyclonal ANCA IgG)**

	PR3-ANCA (n = 9)	MPO-ANCA (n = 11)	anti-GBM (n = 3)	control IgG (n = 5)
<b>Neutrophil (mean ± SEM)</b>				
Degranulation (%)	71.6 ± 4.6	82.3 ± 2.4	52.7 ± 3.0	60.0 ± 7.6
Track Velocity (µm/min)	1.63 ± 0.20	1.81 ± 0.16	2.53 ± 0.39	1.72 ± 0.33
Transmigration (%)	58.0 ± 7.7	58.5 ± 2.6	53.0 ± 12.0	58.2 ± 10.6
Transcellular Transmigration (%)	7.2 ± 2.4	20.2 ± 6.3	10 ± 5.8	27.2 ± 11.9
<b>Monocyte (mean ± SEM)</b>				
Degranulation (%)	63.7 ± 3.3	72.7 ± 4.7	38.3 ± 1.7	67.6 ± 9.2
Track Velocity (µm/min)	0.91 ± 0.11	0.77 ± 0.04	1.05 ± 0.18	0.85 ± 0.06
Transmigration (%)	87.4 ± 3.7	81.1 ± 3.1	81.3 ± 0.7	76.2 ± 9.0
Transcellular Transmigration (%)	29.2 ± 4.8	40.7 ± 4.5	28.2 ± 11.3	40.0 ± 7.0