Supplementary Appendix

This appendix has been provide by the authors to give readers additional information about their work

Supplemental Table 1

MPO-ANCA Cohort (CH): Description of 87 sera samples			
	Number	Mean age ± std (years)	% Female
Clinical Remission	35	61.6 ± 16.8	42.9% (15/35)
Active Disease:	52	53.7 ± 18.8	50% (26/52)
Onset of disease	22	55.8 ± 18.5	50% (11/22)
Relapse	14	61.9 ± 15.6	35.7% (5/14)
Smoldering Active	8	53.1 ± 13.1	87.5% (7/8)
Remission on Therapy	5	70.4 ± 9.3	20% (1/5)
Unresponsive to treatment	3	54.0 ± 21.0	66.7% (2/3)

Supplemental Table 2

MPO-ANCA Cohort (NL): Description of 40 sera samples			
	Number	Mean age ± std (years)	% Female
Clinical Remission	20	66.2 [41.7-78.4]	50% (10/20)
Active Disease:	20	65.5 [41.5-77.9]	50% (10/20)
Onset of disease	20	65.5 [41.5-77.9]	50% (10/20)
Relapse			
Smoldering Active			
Remission on Therapy			
Unresponsive to treatment			

Follow up data on the UNC cohort (in years):

Median: 3.9425

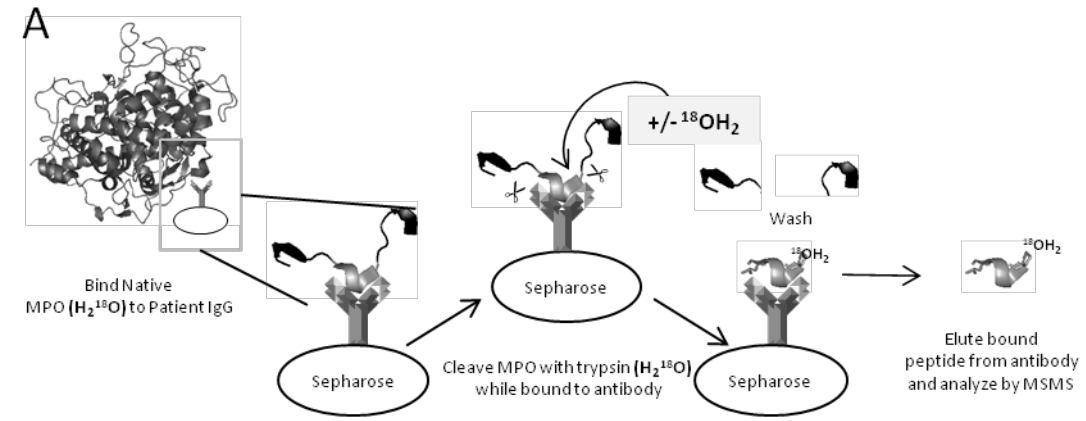
Mean: 5.6867

SD: 5.1586

IQR: 1.2047 to 9.2895

Full range: 0.0301 to 27.8768

The median follow up for the cohort was 3.9 years with 50% of patients followed from 1.2 to 9.3 years (full range was a few days (for those who died early) to as long as 27.9 years).



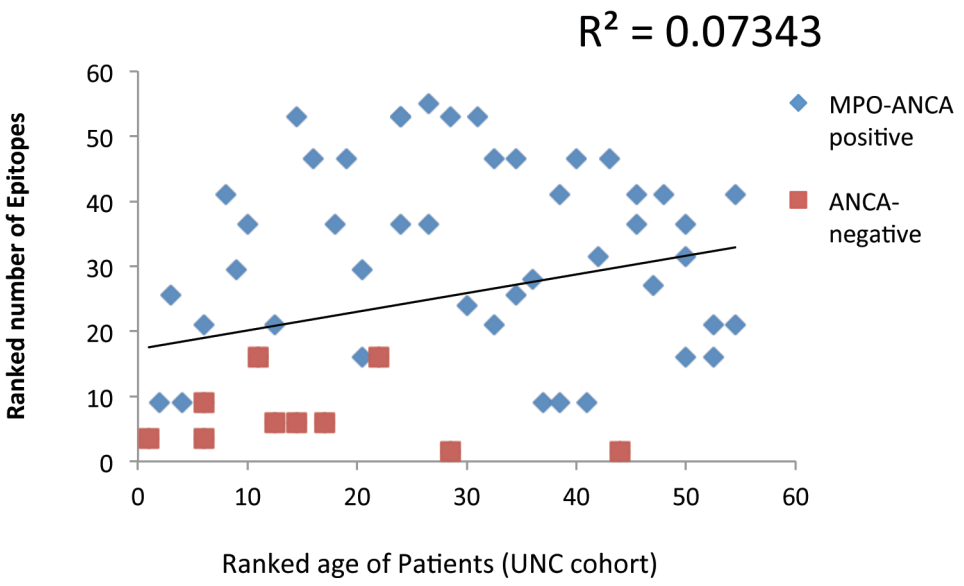
Epitopes associated with disease | **Epitopes not associated with disease**

		537-548	490-499	328-351	220-228	198-219	448-459	715-725	369-374	442-447	657-664	184-193	516-524	560-571	692-701	474-480	437-441	396-405	579-590	237-244	460-473	530-536	593-603	572-578	678-691
Active ANCA-negative patients	1						+												+	+		+			
	2						+																		
	3						+													+		+			
	4						+													+					
	5						+												+	+		+			
	6						+												+						
	7						+																		
	8						+														+				
Clinical Remission	9-11	zero epitopes observed																							
	12																			⊕					
Healthy Subjects	A																					⊕			
	B																								
	C																					⊕			
	D																						⊕		
	E																		⊕		⊕				
	F																				⊕	⊕	⊕		
	G																		⊕		⊕	⊕			
	H												⊕							⊕				⊕	
	I																		⊕		⊕	⊕			⊕
	J																		⊕		⊕	⊕	⊕		⊕

Supplemental Figure 1.

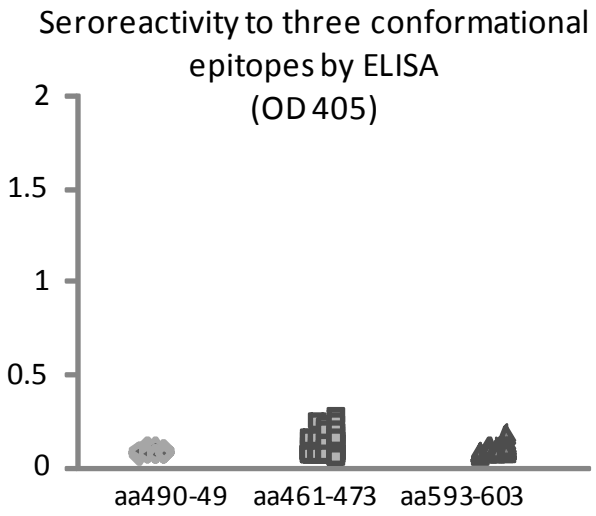
Supplemental Figure 1: Epitope Excision method for conformational epitope mapping of myeloperoxidase (MPO) and identification of MPO specific epitopes in active disease, clinical remission and healthy subjects. **Panel A** shows the epitope excision method with or without the addition of ^{18}O to determine the specificity of autoantibodies to native MPO. **Table:** ANCA-negative patient's sera samples were analyzed at active disease (n=8) and four patients with longitudinal samples during disease remission. These results show that MPO epitope aa447-459 was the exclusive disease associated epitope in this cohort of patients. In comparison ANCA-negative patients have measurable levels of asymptomatic or 'natural' autoantibodies found in healthy controls (circle).

Supplemental Figure 2.



Supplemental Figure 2: Wilcoxon signed-rank test comparing the number of eptiope specific autoantibodies with the age of the individual at the time of sample.

Supplemental Figure 3.



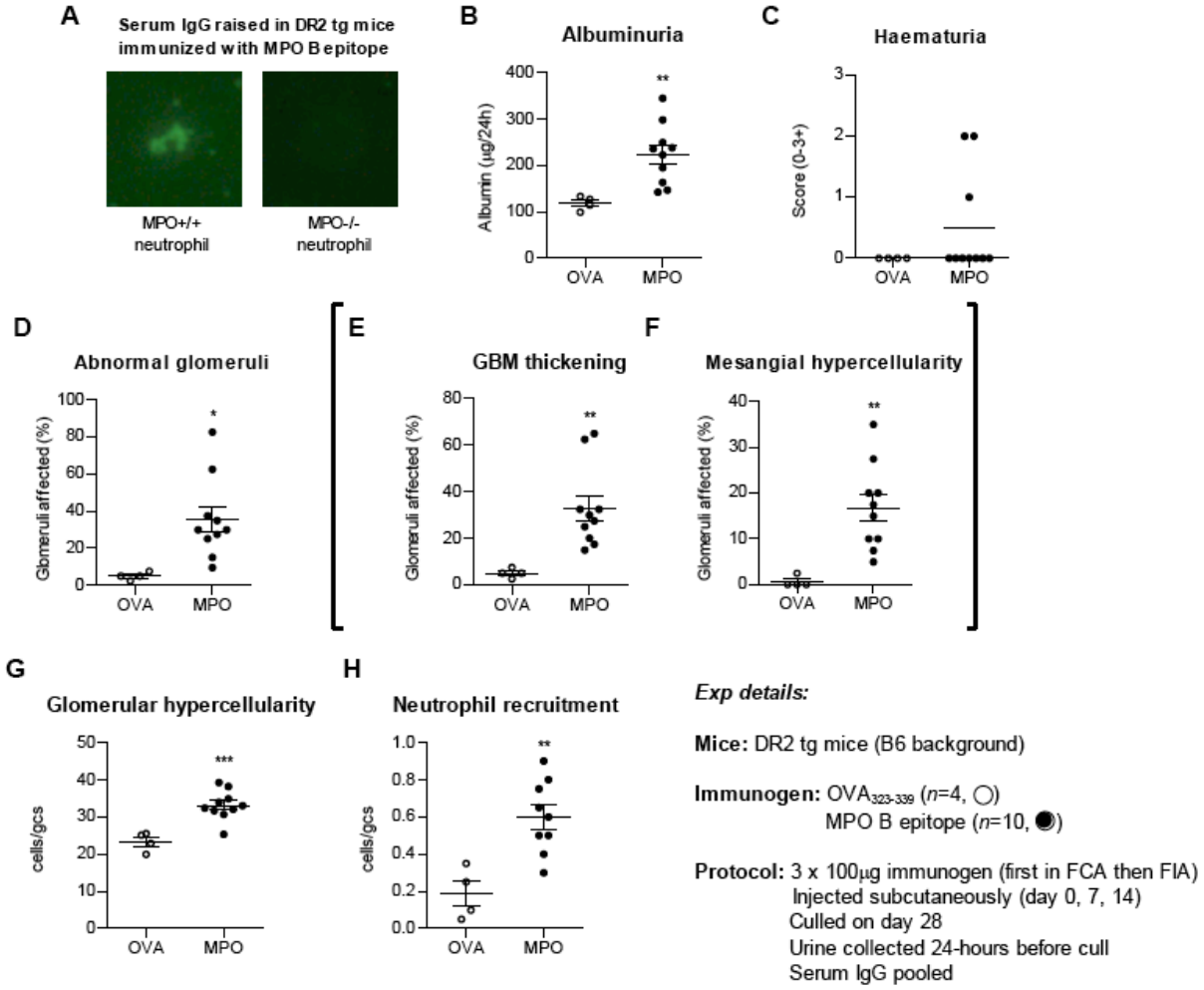
Supplemental Figure 3: Conformationally dependent MPO epitopes, determined by loss of reactivity with pre-digested protein during epitope excision MS protocol, were synthesized as peptides and tested for reactivity by ELISA.

Supplemental Figure 4: Fine Epitope Mapping

Trypsin Cut (RK)	Chymotrypsin Cut (FYWLM)
NGFPVALAR	NGFPVALAR
FPTDQLTPDQER	FPTDQLTPDQER
FQDNGR	FQDNGR
RKIVGAMVQIITYR	RKIVGAMVQIITYR
IANVFTNAFR	IANVFTNAFR
VFFASWR	VFFASWR
QNQIAVDEIR	QNQIAVDEIR
IGLDLPALNMQR	IGLDLPALNMQR
QALAQISLPR	QALAQISLPR
YQPMEPNPR	YQPMEPNPR
DHGLPGYNAWR	DHGLPGYNAWR
DYLPLVLGPTAMR	DYLPLVLGPTAMR

Supplemental Figure 4: MS epitope excision was used to fine epitope map MPO epitopes by substituting the tryptic digest with chymotrypsin. This alternate analysis shows the specific amino acids (shown in red and blue) that remain bound to the antibody using a more aggressive enzymatic digestion.

Supplemental Figure 5: DR2 tg mice immunized with murine MPO epitope aa442-460 develop GN



Exp details:

Mice: DR2 tg mice (B6 background)

Immunogen: $OVA_{323-339}$ (*n*=4, ○)
MPO B epitope (*n*=10, ●)

Protocol: 3 x 100µg immunogen (first in FCA then FIA)
Injected subcutaneously (day 0, 7, 14)
Culled on day 28
Urine collected 24-hours before cull
Serum IgG pooled

Fig. Legends:

(A) Indirect immunofluorescence of thioglycollate induced peritoneal neutrophils using pooled serum IgG of DR2 tg mice immunized with the MPO B epitope. (IgG concentration for staining: 1mg/ml).

(B) Albuminuria measured by ELISA on urine collected 24 hours before the end of the experiment.

(C) Haematuria measured by urine test strips.

(D, E and F) Abnormal glomeruli was assessed on formalin fixed PAS stained kidneys. Abnormalities assessed were thickening of the GBM (E) and evidence of mesangial hypercellularity (three or more attached nuclei) (F). Necrosis was rare.

(G) Glomerular hypercellularity was assessed by enumerating the number of cells per glomerulus.

(H) Glomerular neutrophil recruitment was assesses by immunohistochemistry by anti-Gr-1 antibody on PLP-fixed frozen kidneys.