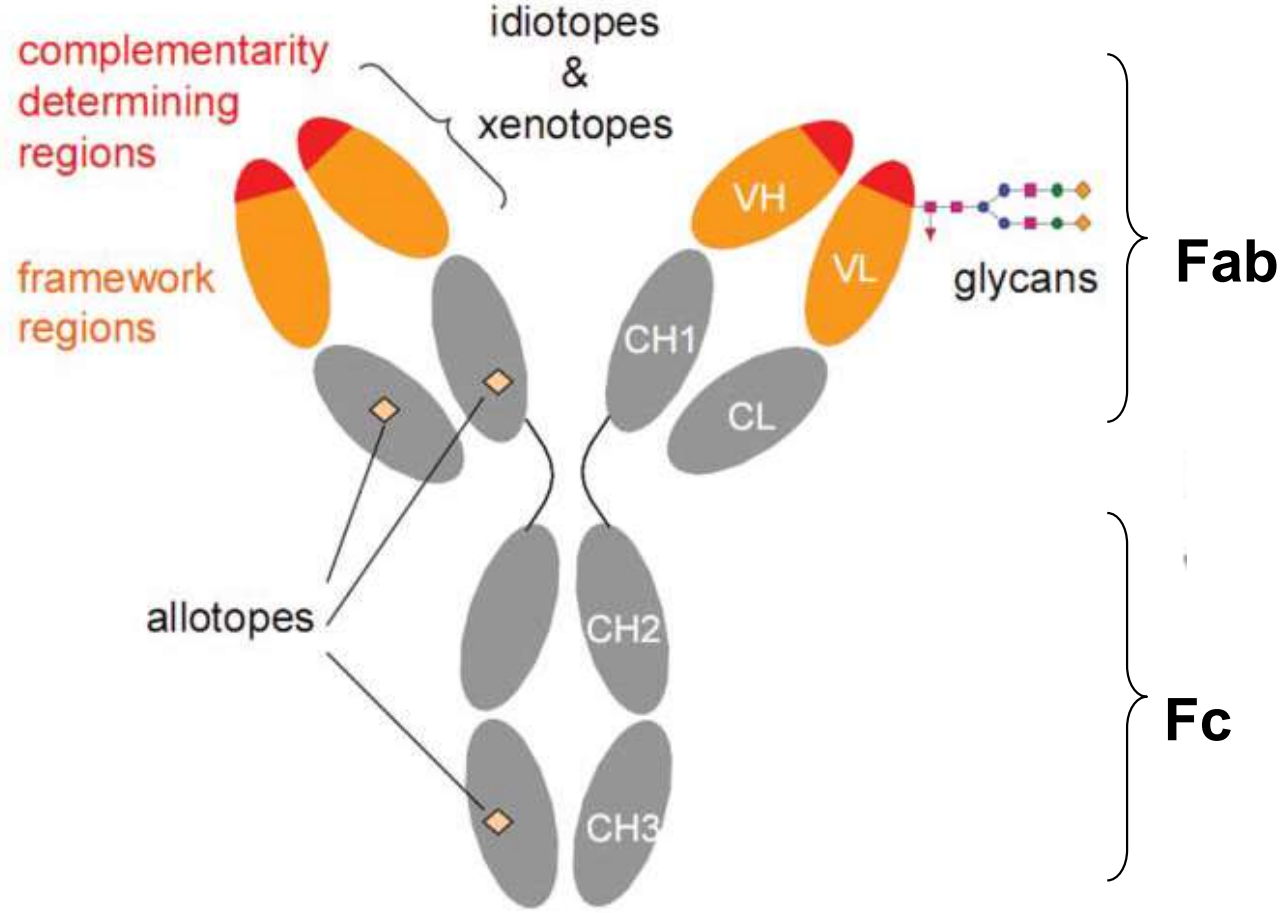
Antibodies to constant domains of IgG: Dissecting the Rheumatoid Factor

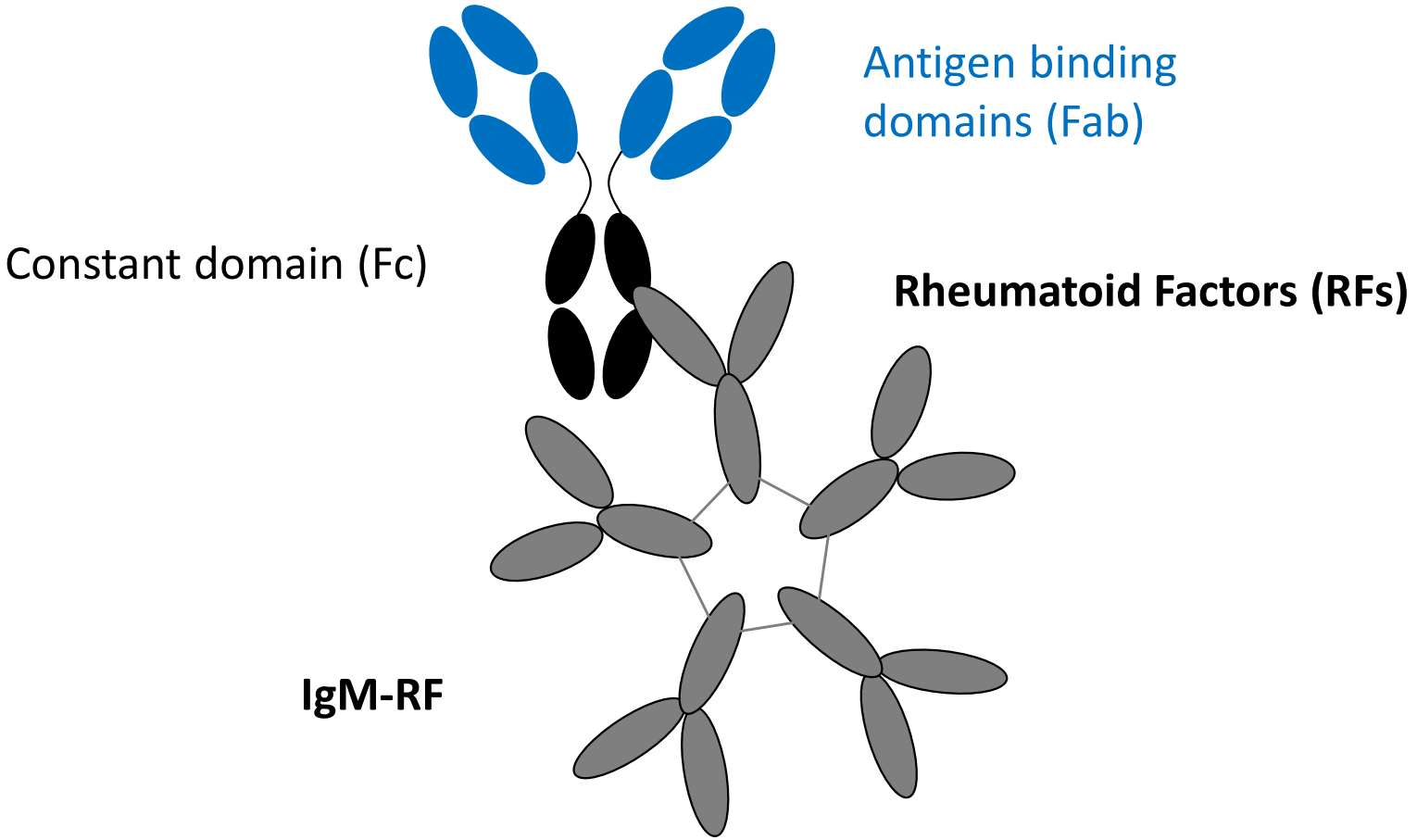
Antibodies as antigens

- Alloreactivity (mother/child; blood transfusions)
- Autoreactivity (‘Rheumatoid factors’)
- Immunogenicity of therapeutic monoclonal antibodies



Van Schie et al. mAbs 2015

Autoantibodies binding to IgG: Rheumatoid Factors

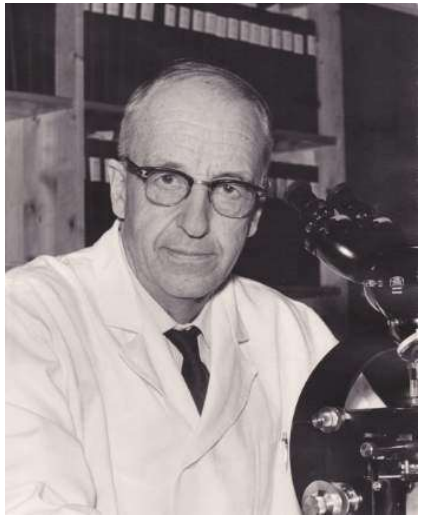


RF (and ACPA) are used in diagnosing RA

Table 3. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis

	Score
Target population (Who should be tested?): Patients who	
1) have at least 1 joint with definite clinical synovitis (swelling)*	
2) with the synovitis not better explained by another disease†	
Classification criteria for RA (score-based algorithm: add score of categories A–D;	
B. Serology (at least 1 test result is needed for classification)††	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
B. Serology (at least 1 test result is needed for classification)††	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡	
Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms§§	
<6 weeks	0
≥6 weeks	1

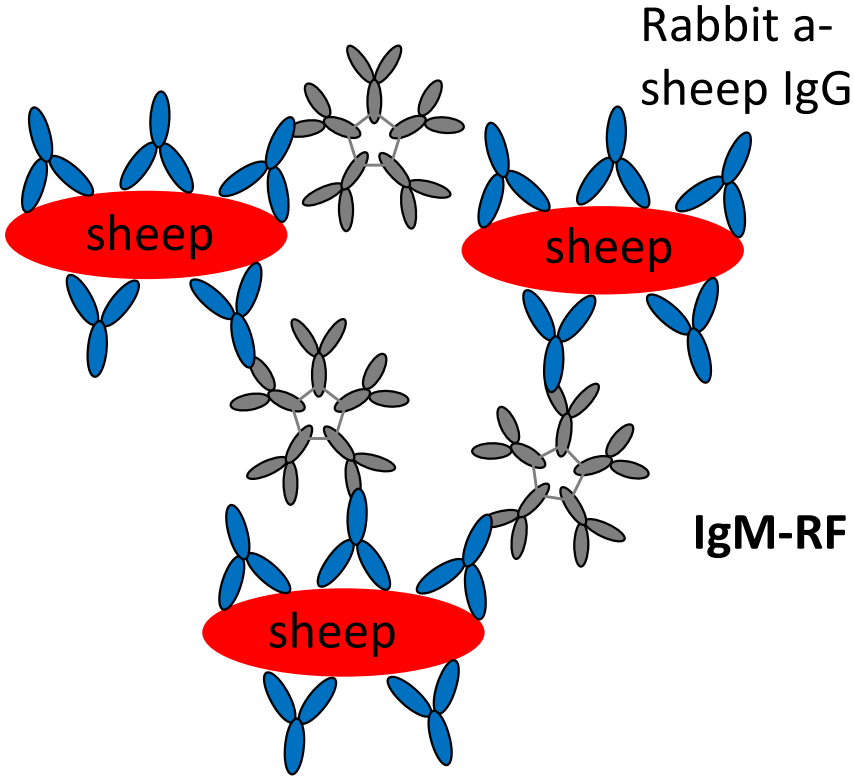
How do conventional RF tests work?



ON THE OCCURRENCE OF A FACTOR IN HUMAN SERUM ACTIVATING THE SPECIFIC AGGLUTINATION OF SHEEP BLOOD CORPUSCLES.

By *Erik Waaler*, M. D.

(Received for publication December 15th, 1939).



Agglutination of sheep red blood cells sensitized with anti-sheep rabbit serum

The "Waaler-Rose reaction"

Limitations of RF testing

RF testing is as sensitive (60%) but less specific than ACPA testing (96% versus 86%)

RF are present in other diseases and 5% of healthy population

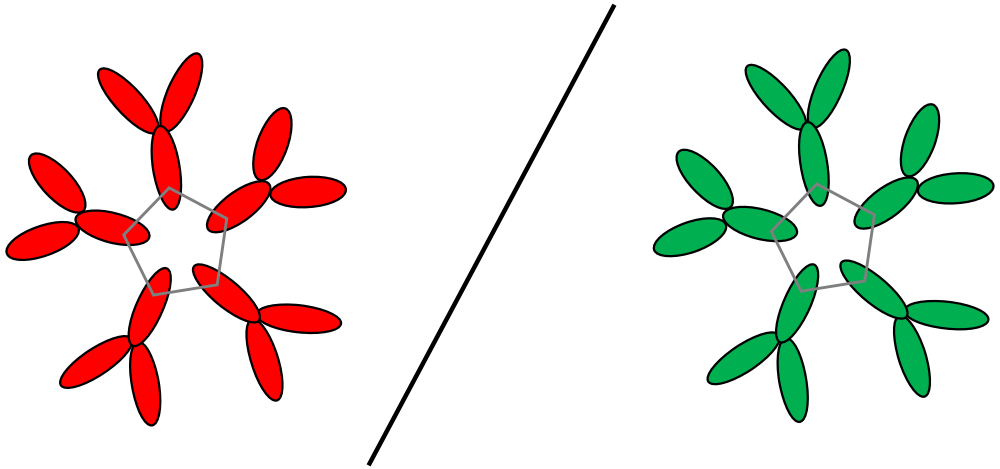
Discrepancies between different RF assays

Table 1. Conditions associated with a positive RF.

Condition	Frequency (%)
Rheumatic diseases	
Rheumatoid arthritis *	50–90
Systemic lupus erythematosus *	15–35
Sjøgren's syndrome *	75–95
Systemic sclerosis *	20–30
Polymyositis/dermatomyositis *	5–10
Cryoglobulinaemia *	40–100
Mixed connective tissue disease *	50–60
Infection	
Bacterial endocarditis *	25–50
Hepatitis B and C *	20–75
Tuberculosis	8
Syphilis *	Up to 13
Parasitic diseases	20–90
Leprosy *	5–58
Viral infection *	15–65
Pulmonary diseases	
Sarcoidosis *	3–33
Interstitial pulmonary fibrosis	10–50
Silicosis	30–50
Asbestosis	30
Miscellaneous diseases	
Primary biliary cirrhosis *	45–70
Malignancy *	5–25
Age > 70 years	5–25

Besada, E., et al. (2012). Scand J Rheumatol

Can we develop a new RF assay to distinguish “disease-associated” from “physiological” RF responses?



→ Better prediction of disease onset and disease course

→ Use this to make better treatment decisions

Mapping IgG Epitopes Bound by Rheumatoid Factors from Immunized Controls Identifies Disease-Specific Rheumatoid Factors Produced by Patients with Rheumatoid Arthritis[1]

Vincent R. Bonagura,[2]*† Nick Agostino,* Marie Børretzen,‡ Keith M. Thompson,‡ Jacob B. Natvig,‡ and Sherie L. Morrison§

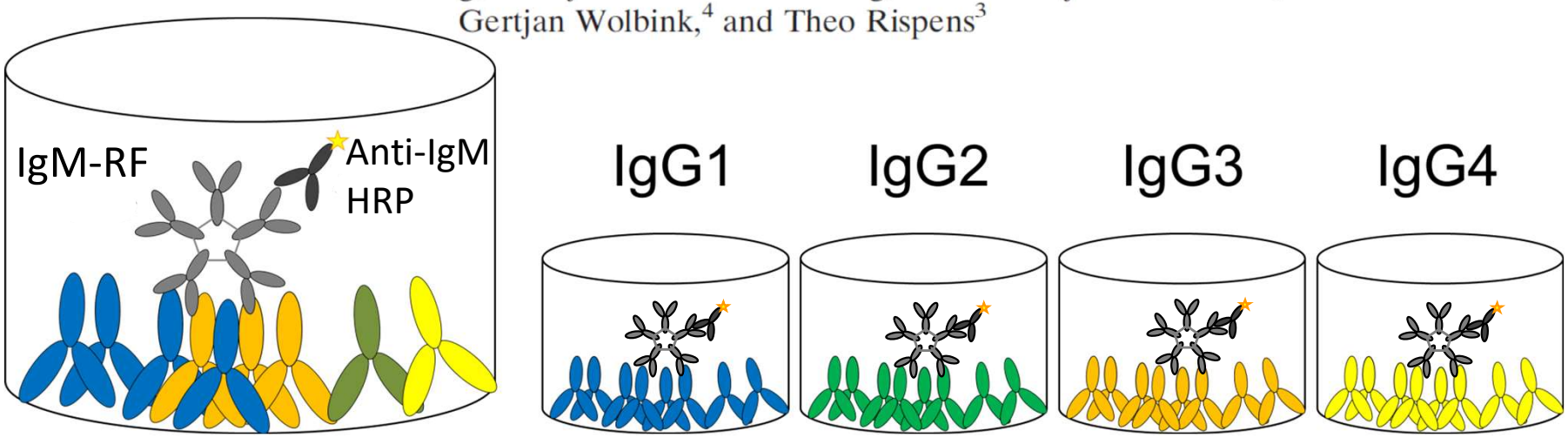
Table VIII. *Summary of RF binding specificities[a], V_H and V_L usage[a]*

	Ala[b] 435	4443[c] His[435]	3333 His[435]	Ala[b] 253	Pro[b] 252-4	Gly[b] 252-4	Pro[b] 309-11	Gly[b] 309-11	Inhibit[d] by SPA	V_L	V_H
GA pattern											
MR-1	−	+	−	−	−	−	+	+	+	325	V_H1
MR-2[e]	−	+	−	−	−	−	+	+	+	325	V_H1
MR-3	−	+	−	−	−	−	+	+/−	+	K3	V_H1
MR-5	−	+	−	−	−	−	−	−	+	328	V_H3
MR-12	−	+	−	−	−	−	+	−	+	K3	ND
MR-13[e]	−	+	−	−	−	−	+	−	+	325	V_H1
MR-14[e]	−	+	−	−	−	−	+/−	−	+/−	325	V_H1
MR-20	−	+	−	−	−	−	+	+	+	K3	V_H4
MR-25[e]	−	+	−	−	−	−	+	−	+	325	V_H1
MR-27	−	+	−	−	−	−	−	+	+	ND	V_H3
MR-28[e]	−	+	−	−	−	−	+	−	+	325	V_H1
MR-30[e]	−	+	−	−	−	−	+	−	+	325	V_H1
MR-33[e]	+	+	−	−	−	−	+	+	+	325	V_H1
MR-37[e]	−	+	−	−	−	−	+	−	+	325	V_H1
MR-39	−	+	−	−	−	−	+	+	+	K3	V_H4
MR-41	−	+	−	−	−	−	−	−	−	328	V_H3
DI-2	−	+	−	−	−	−	+	+	−	K3	V_H3
FO-3	−	+	+	−	−	−	−	−	+	λ	V_H4

RF testing with IgG subclasses as targets

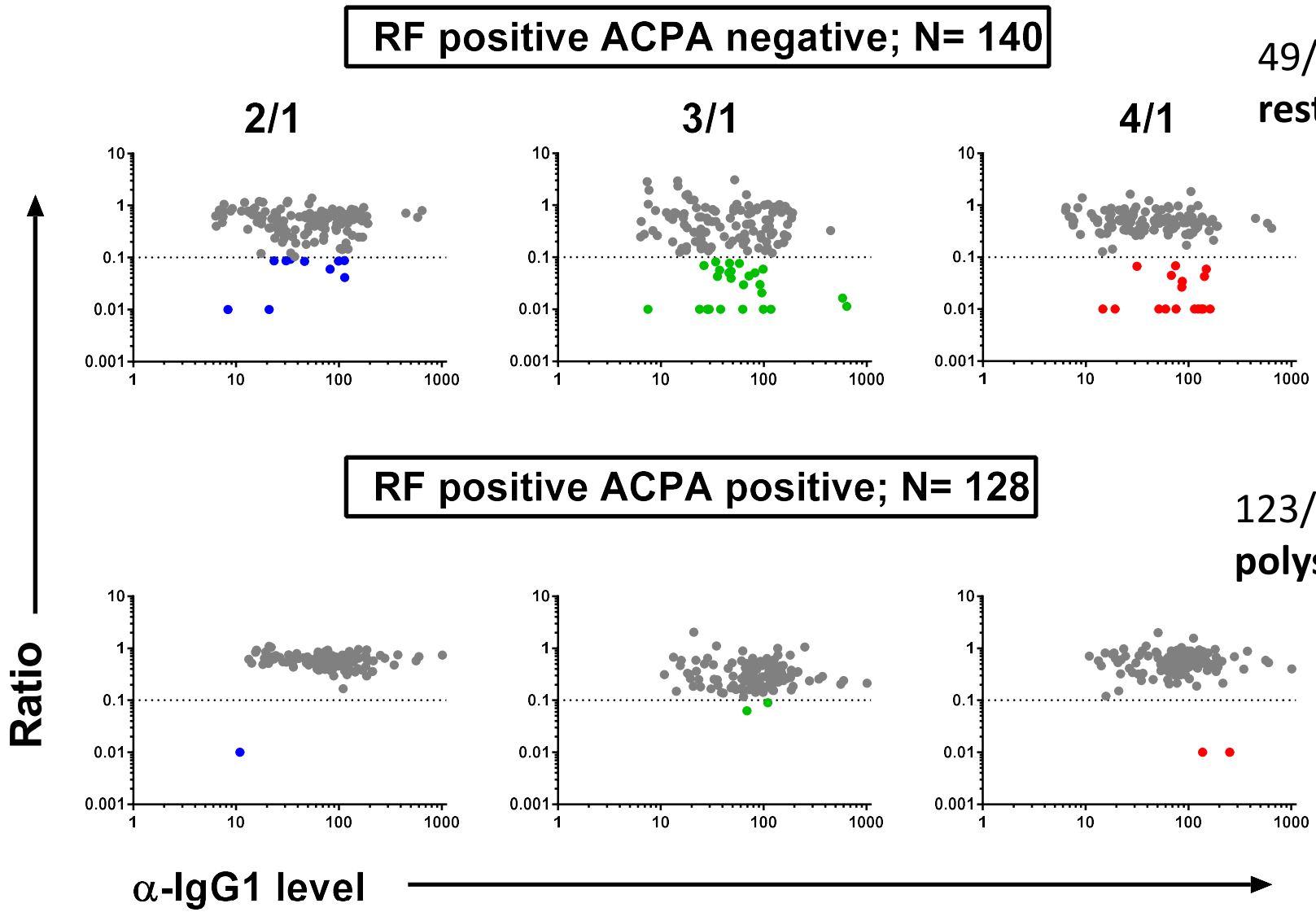
IgG Subclass Specificity Discriminates Restricted IgM Rheumatoid Factor Responses From More Mature Anti–Citrullinated Protein Antibody–Associated or Isotype-Switched IgA Responses

Willem J. J. Falkenburg,[1] Dirkjan van Schaardenburg,[2] Pleuni Ooijevaar-de Heer,[3] Gertjan Wolbink,[4] and Theo Rispens[3]



Polyspecific RF response →	+	+	+	+
Restricted RF response	+	-	+	+
	+	+	-	+
	+	+	+	-

Restricted RF responses almost exclusively in the ACPA negative group



49/140 (35%) have a **restricted** RF response

123/128 (96%) have a **polyspecific** RF response

Adapted from: Falkenburg et al. (2015). Arthritis Rheumatol

Binding of human IgM from a rheumatoid factor to IgG of 12 animal species

Jiharu Hamako,*† Yasuhiro Ozeki,* Taei Matsui,* Yoshinobu Yamamoto,† Takashi Inoue,‡ Jun Yukitake§ and Koiti Titani*

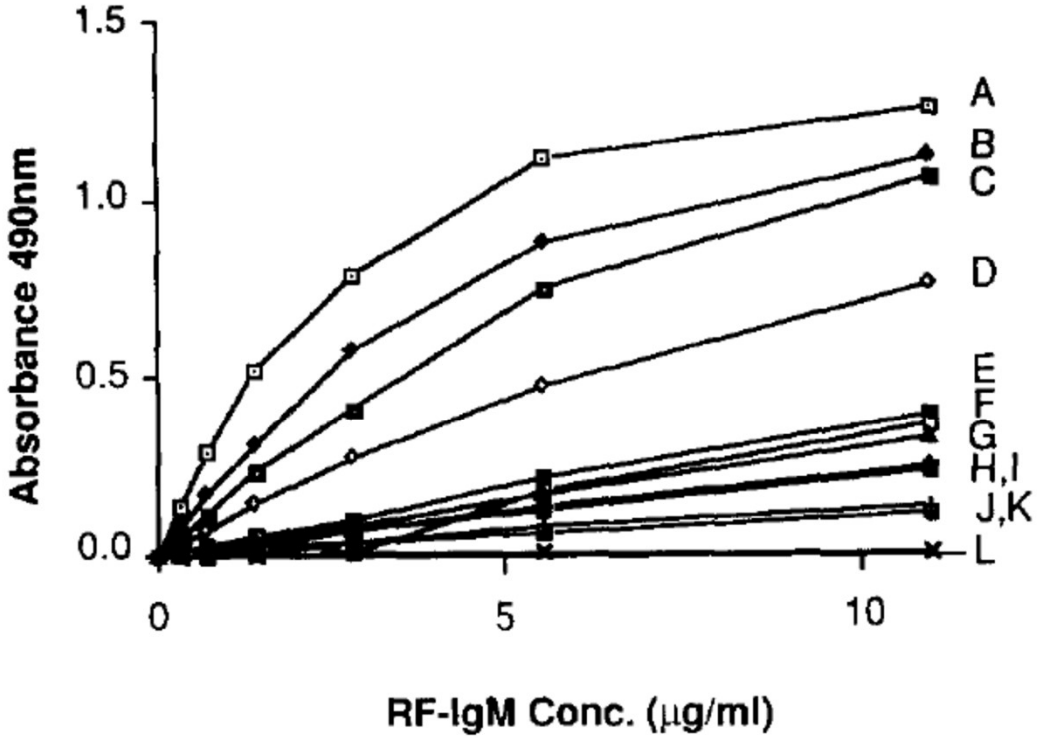
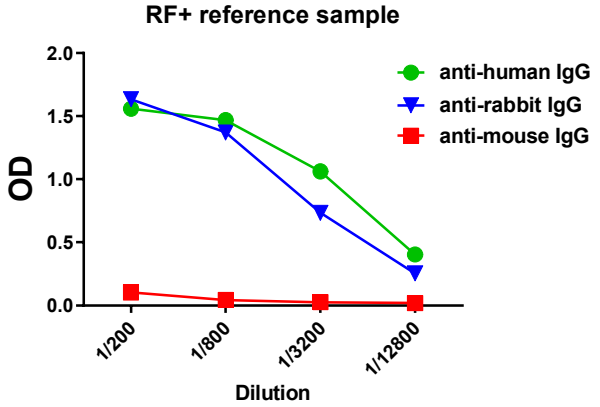


Fig. 2. Binding of IgM derived from RF-positive serum to 12 animal IgG measured by ELISA. One hundred microliters of RF-IgM at various concentration was applied to a plate coated with each IgG (150 ng/well) and incubated at room temperature for 1 hr. Binding of IgM to IgG was detected with HRP-conjugated anti-human IgM antibody. Source of IgG: A, human; B, rabbit; C, horse; D, pig; E, cow; F, sheep; G, goat; H, guinea pig; I, dog; J, mouse; K, rat; L, chicken.



Predicting relevant RF epitopes on IgG-Fc

```
CH2
Homsap IGHG1   APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
Musmus IGHG2B  APNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTIRVVSTLPIQHQDWMSGKEFKCKVNNKDLPSPIERTISKIK
Orycun IGHG    PPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSEDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIAHEDWLRGKEFKCKVHNKALPAPIEKTISKAR
                 x x          xx  x oooo            y      x  x             xx   o           ooo   x                y
                 o o          11  1 AAA2                   3  4             55   B           CCC   6
CH3
Homsap IGHG1   GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Musmus IGHG2B  GLVRAPQVYTLPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLNMKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK
Orycun IGHG    GQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK
                xx x        *o  x              x x                                 x     x     xo  o    x x  x  x  ooxx      0
                77 8         9  10             1112                                13    14    15  16   17   18 19 20 21      22
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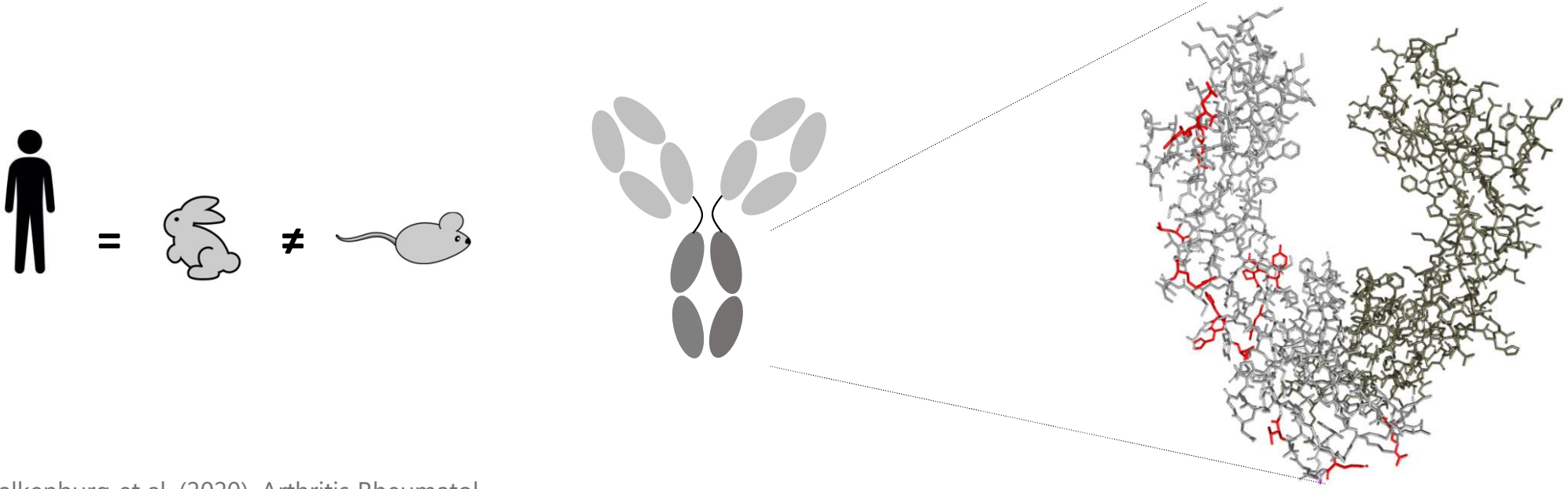
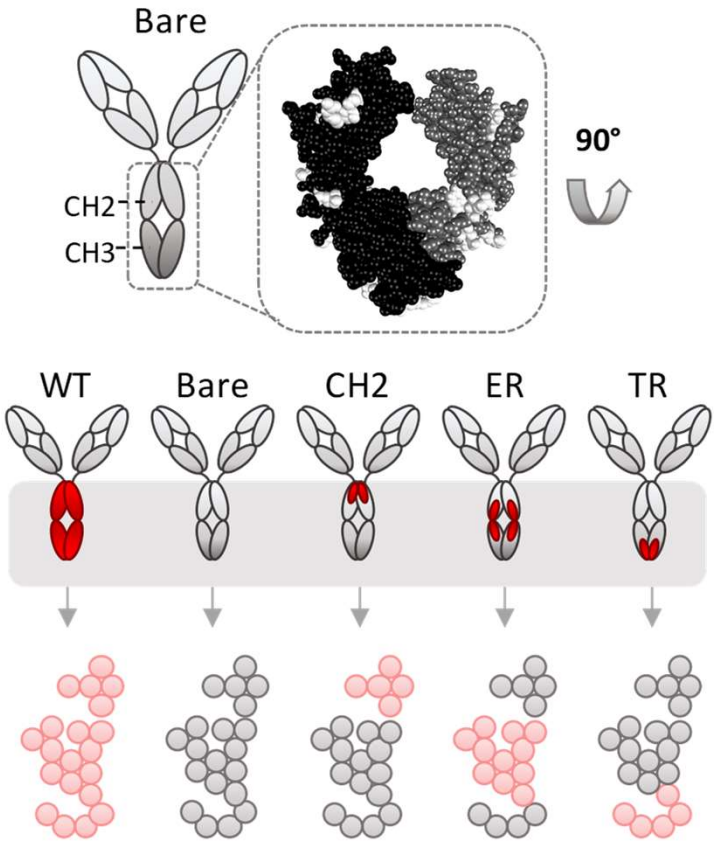
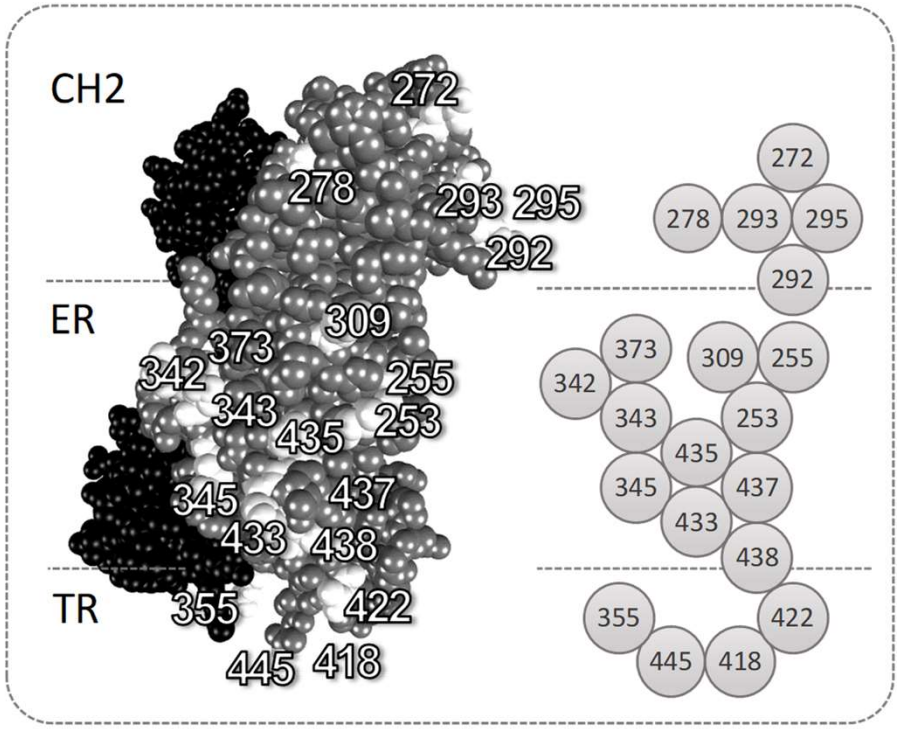


Falkenburg et al. (2020). Arthritis Rheumatol

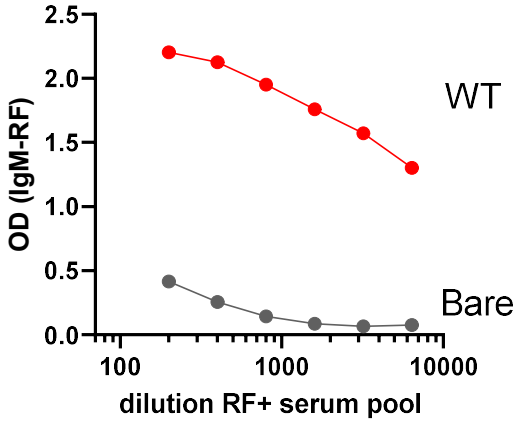
Comprehensive mapping - strategy





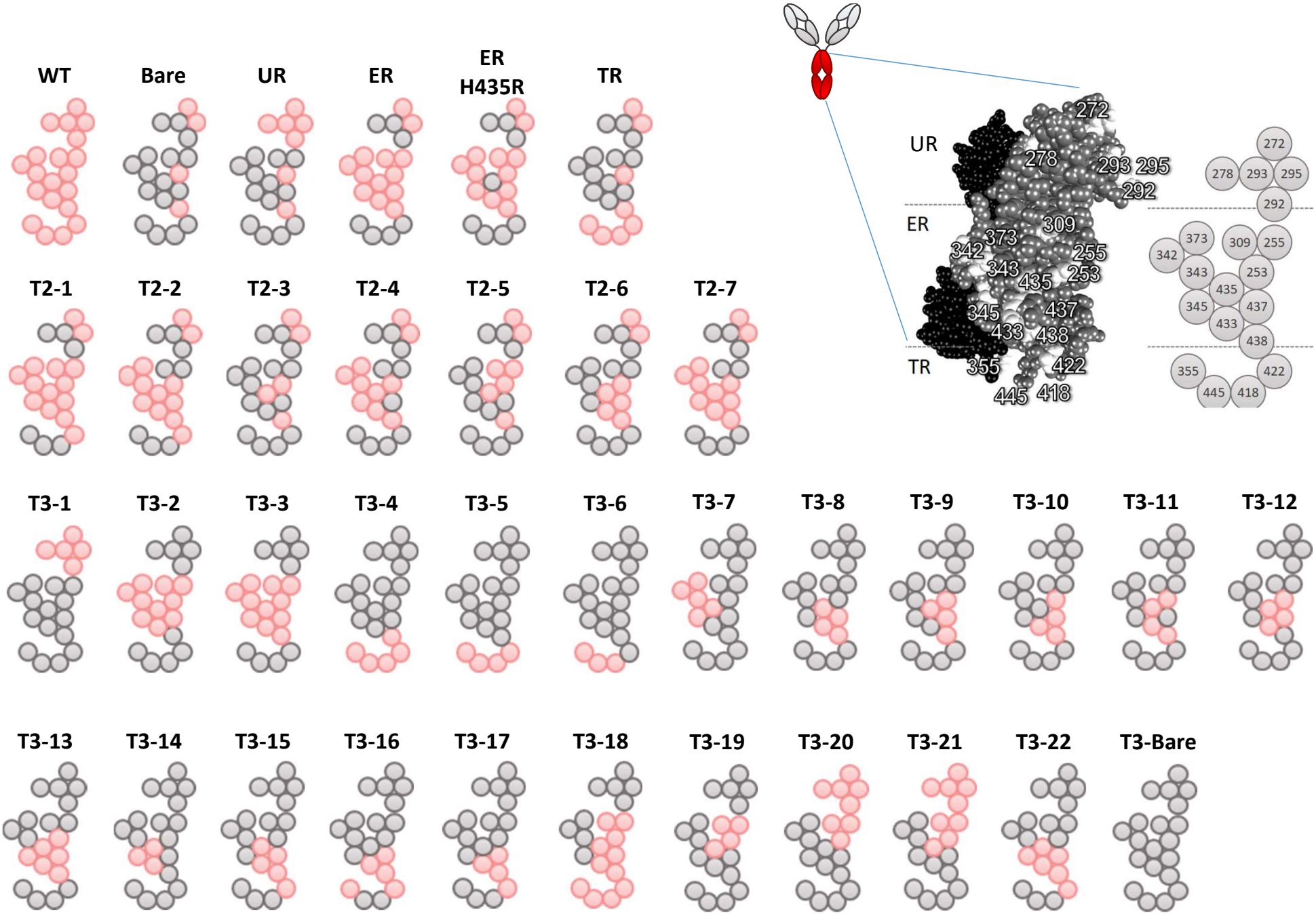
Mouse / no RF binding

Human / RF binding



Oskam et al. Ann Rheum Dis 2023

Exploring RF reactivity patterns: targets



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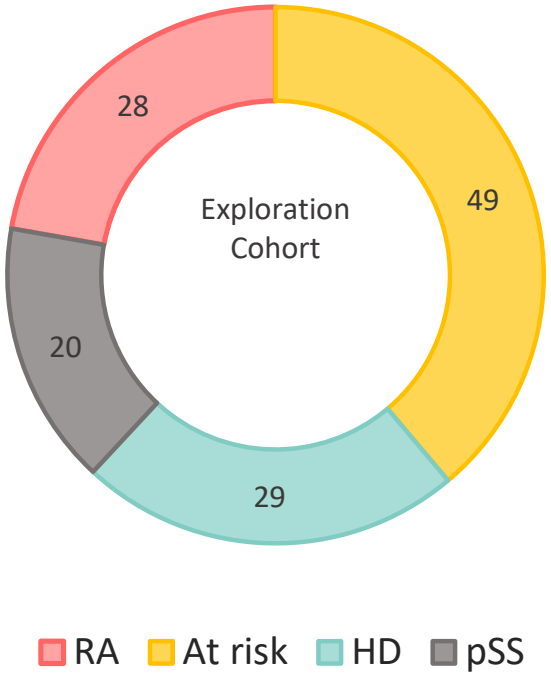
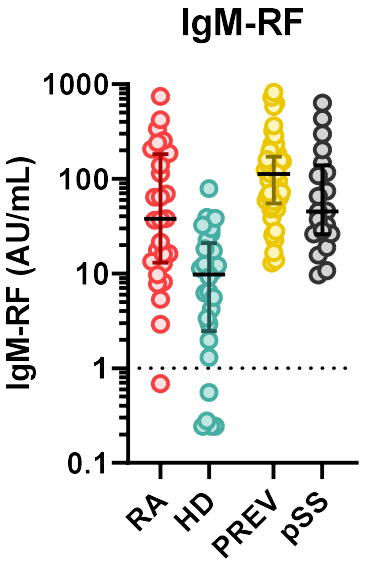
Exploring RF reactivity patterns





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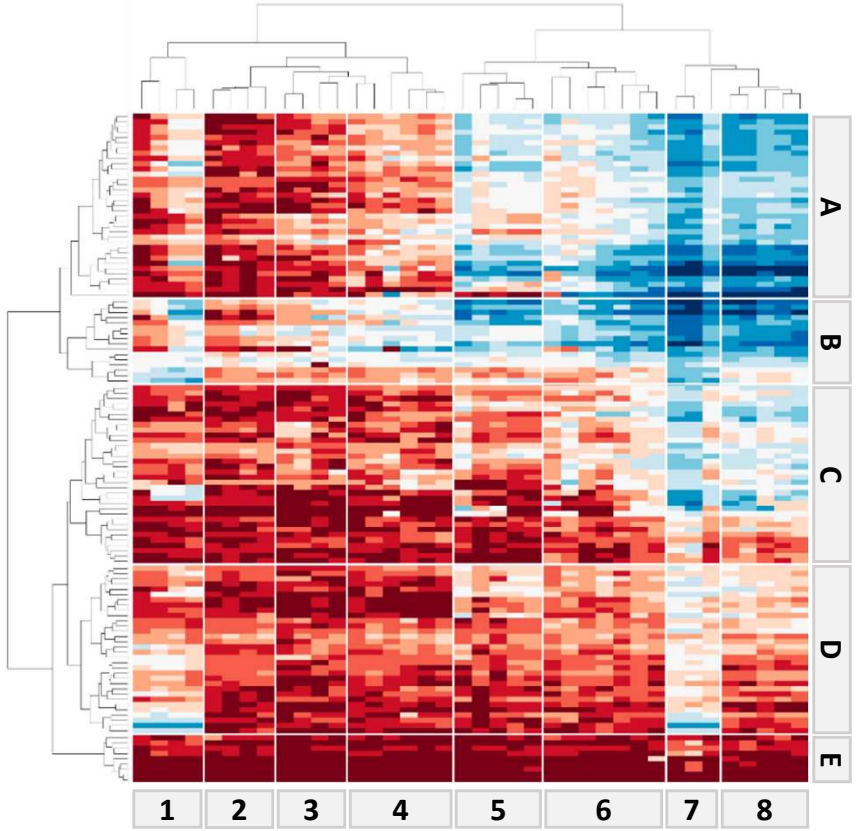
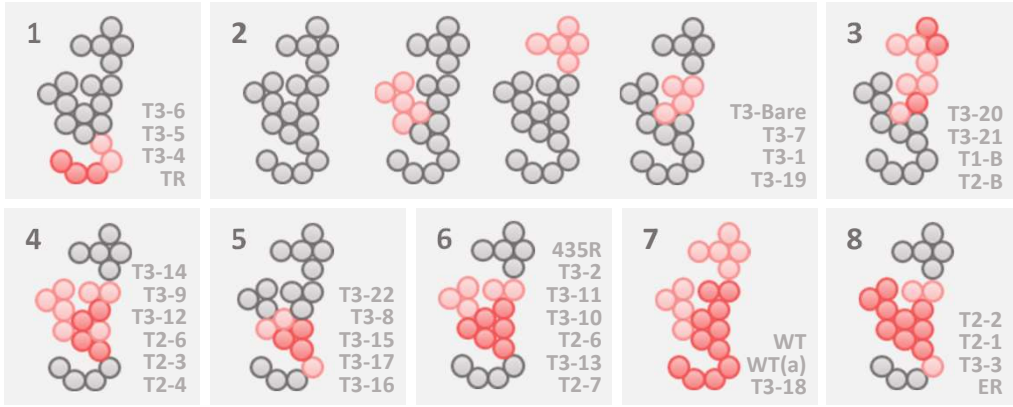
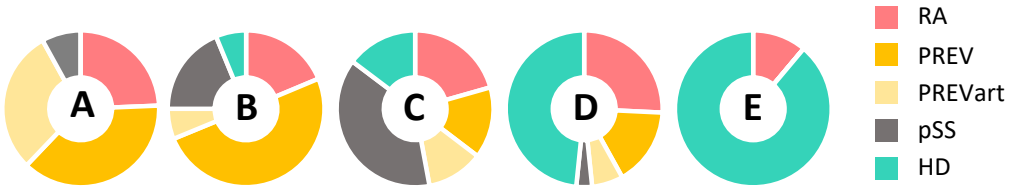
Exploring RF reactivity patterns







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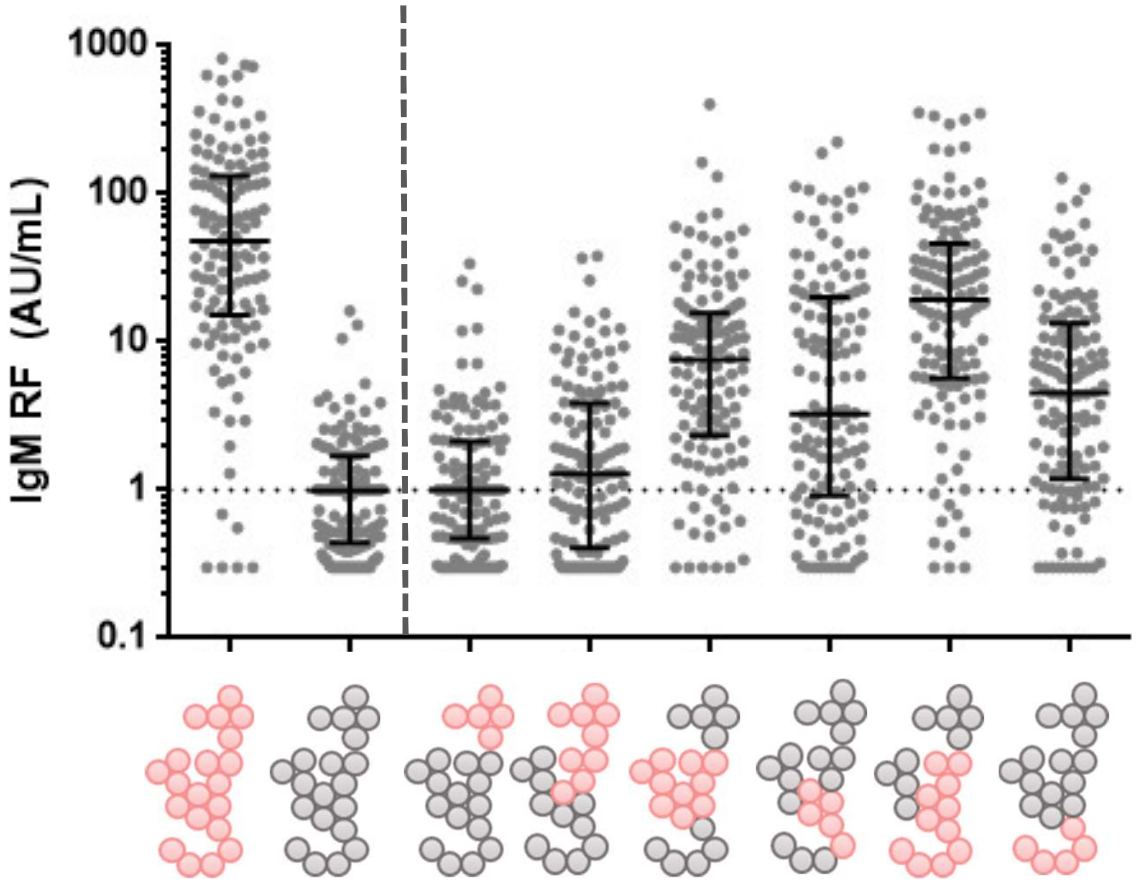
RF reactivity across the Fc



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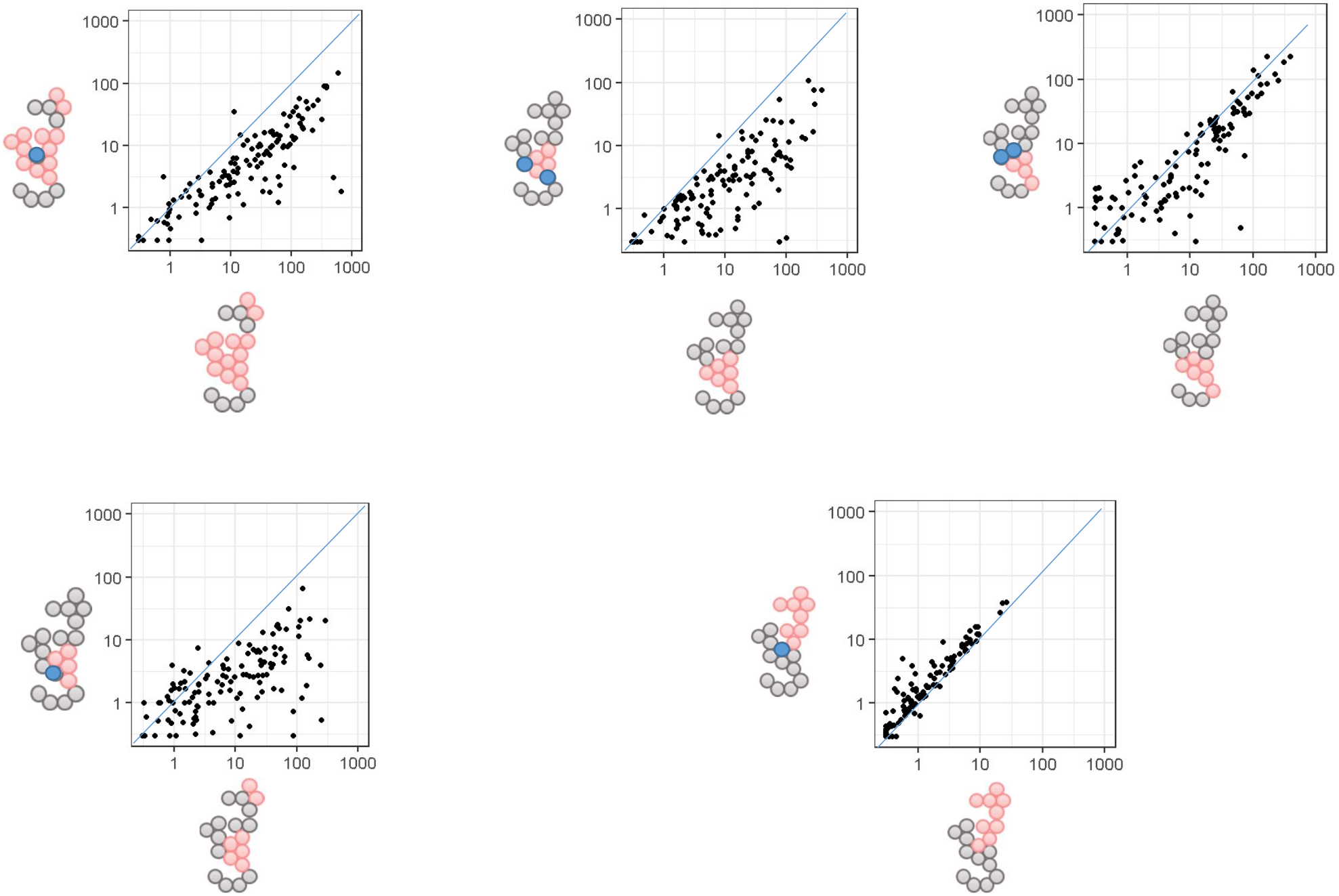
Fine specificities



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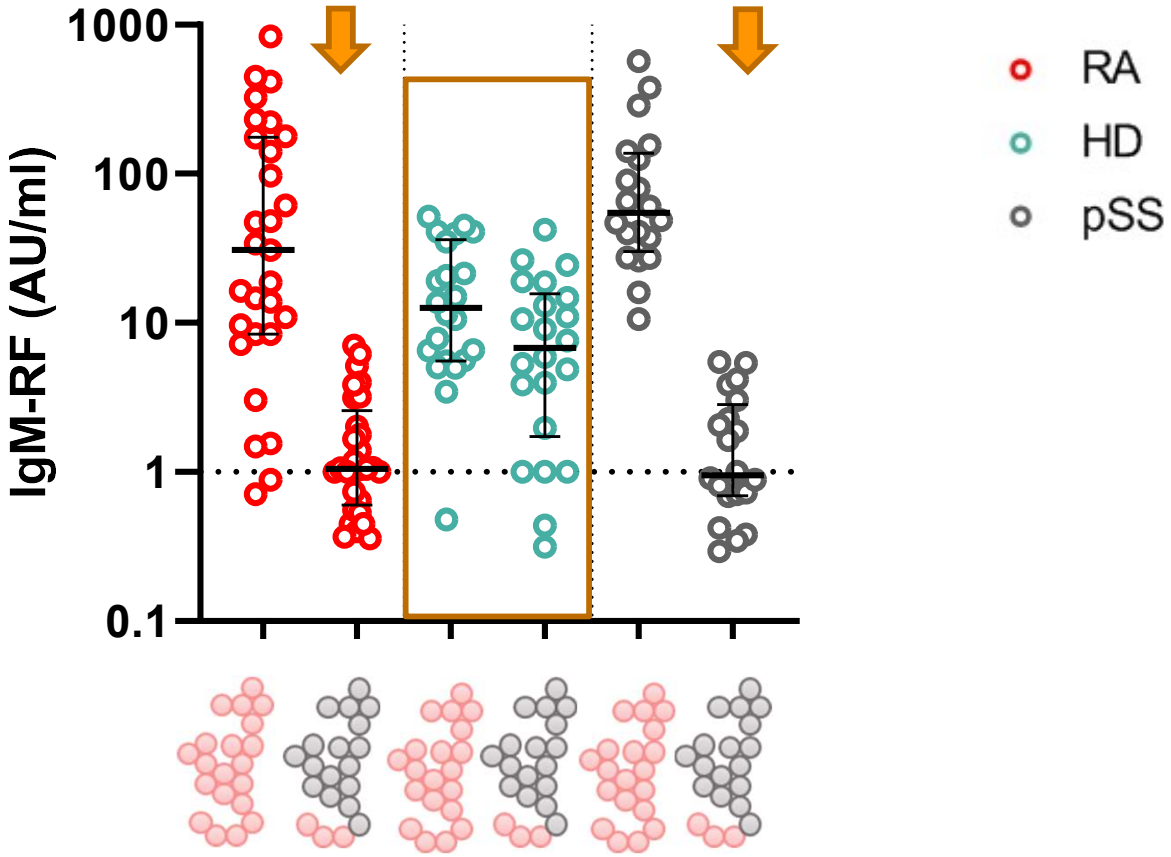
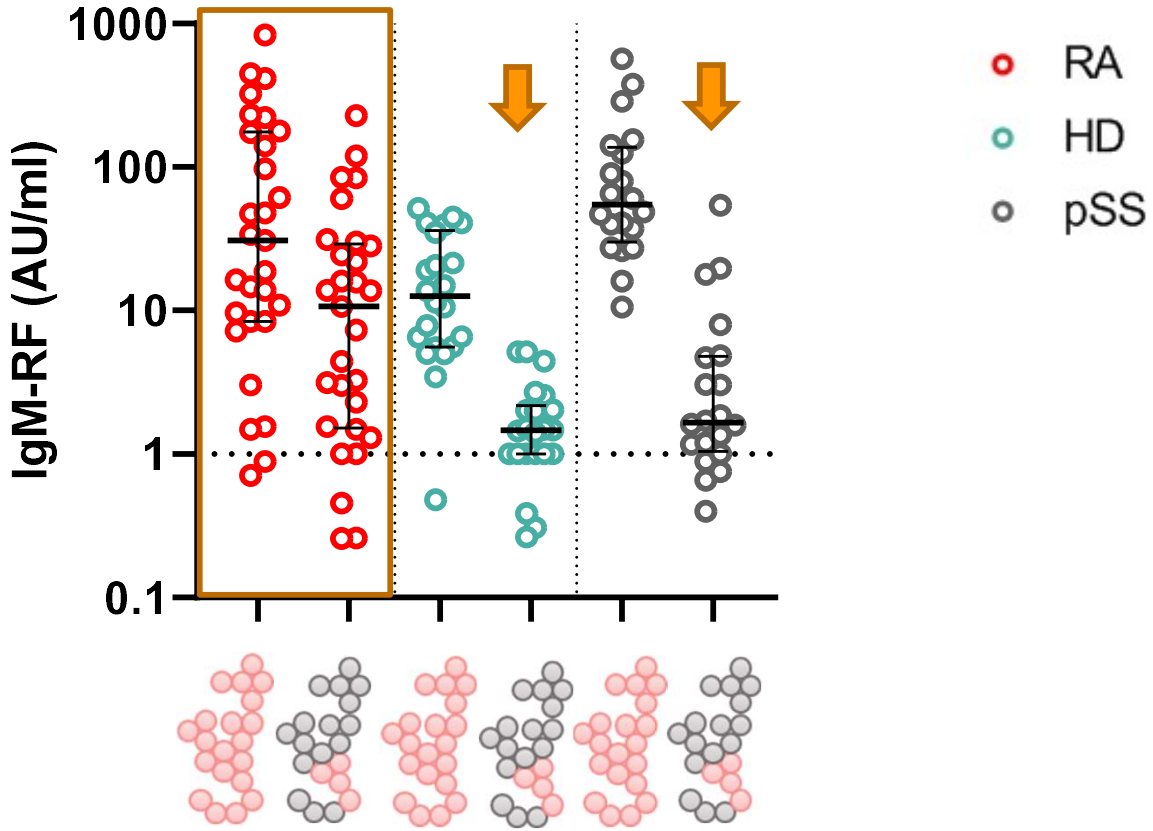
RF in healthy individuals



RF in RA patients



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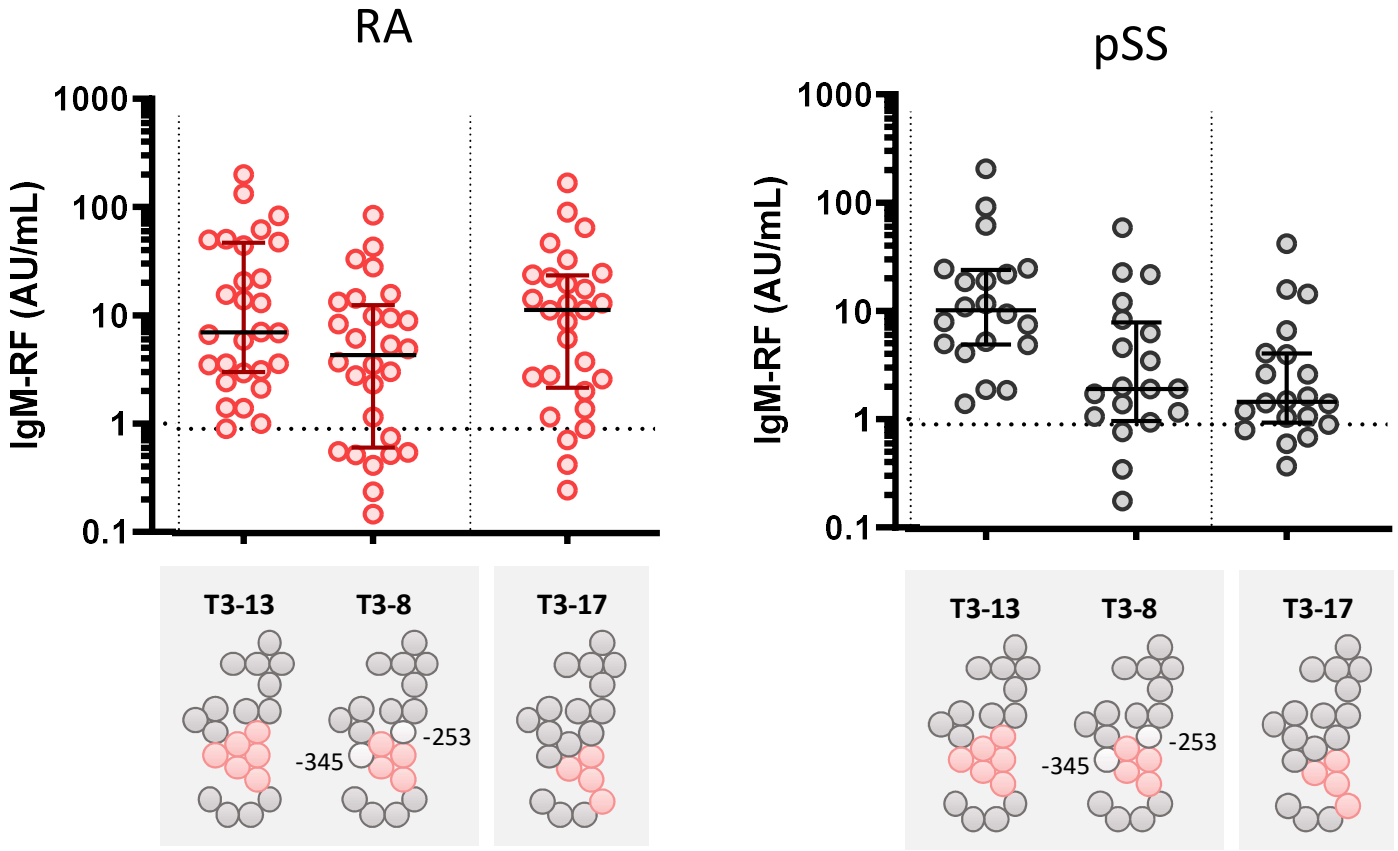
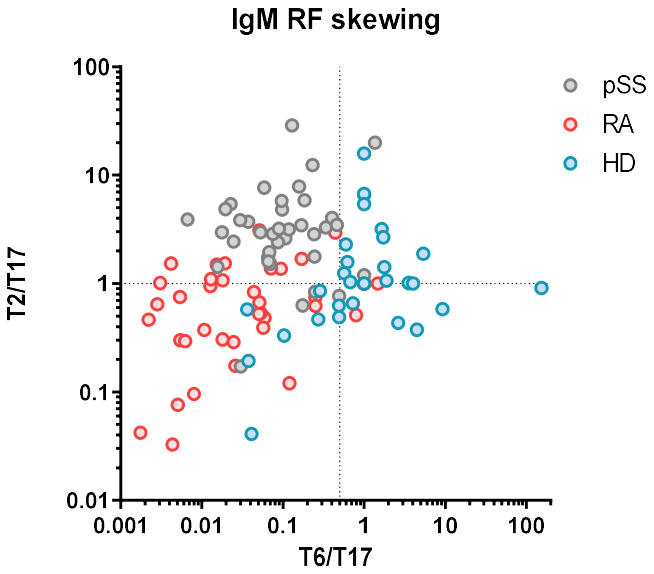
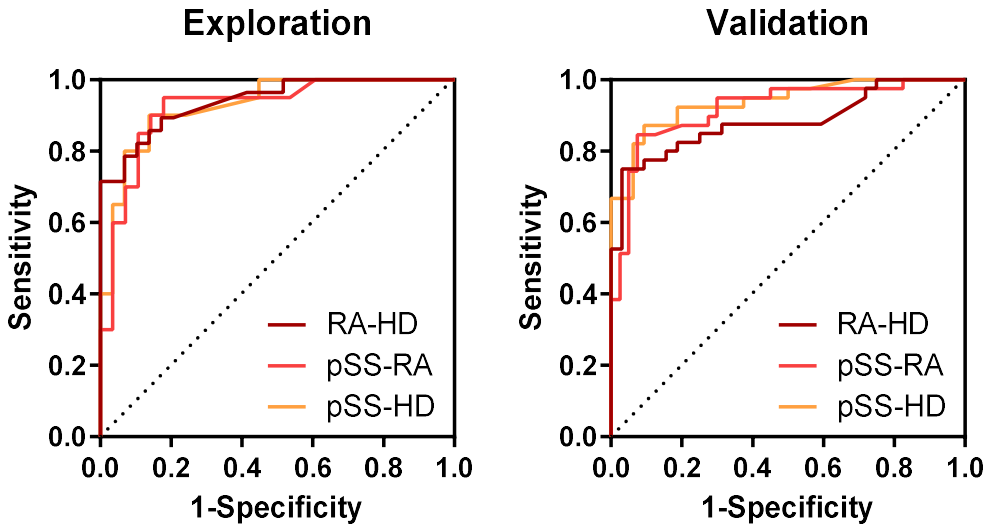
RF in pSS patients



Distinct patterns using minimal target set





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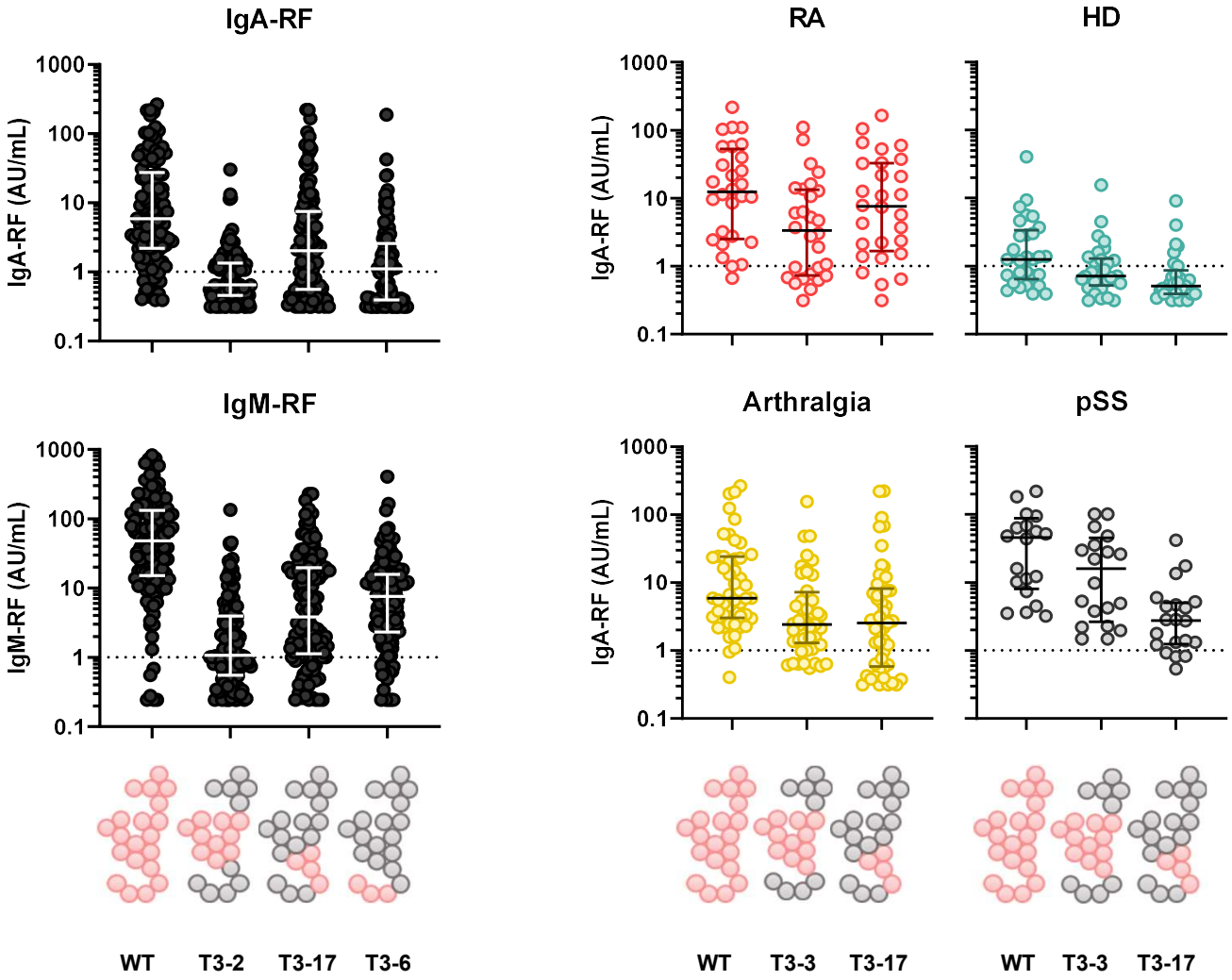
IgA-RF binding patterns restricted to fewer epitopes



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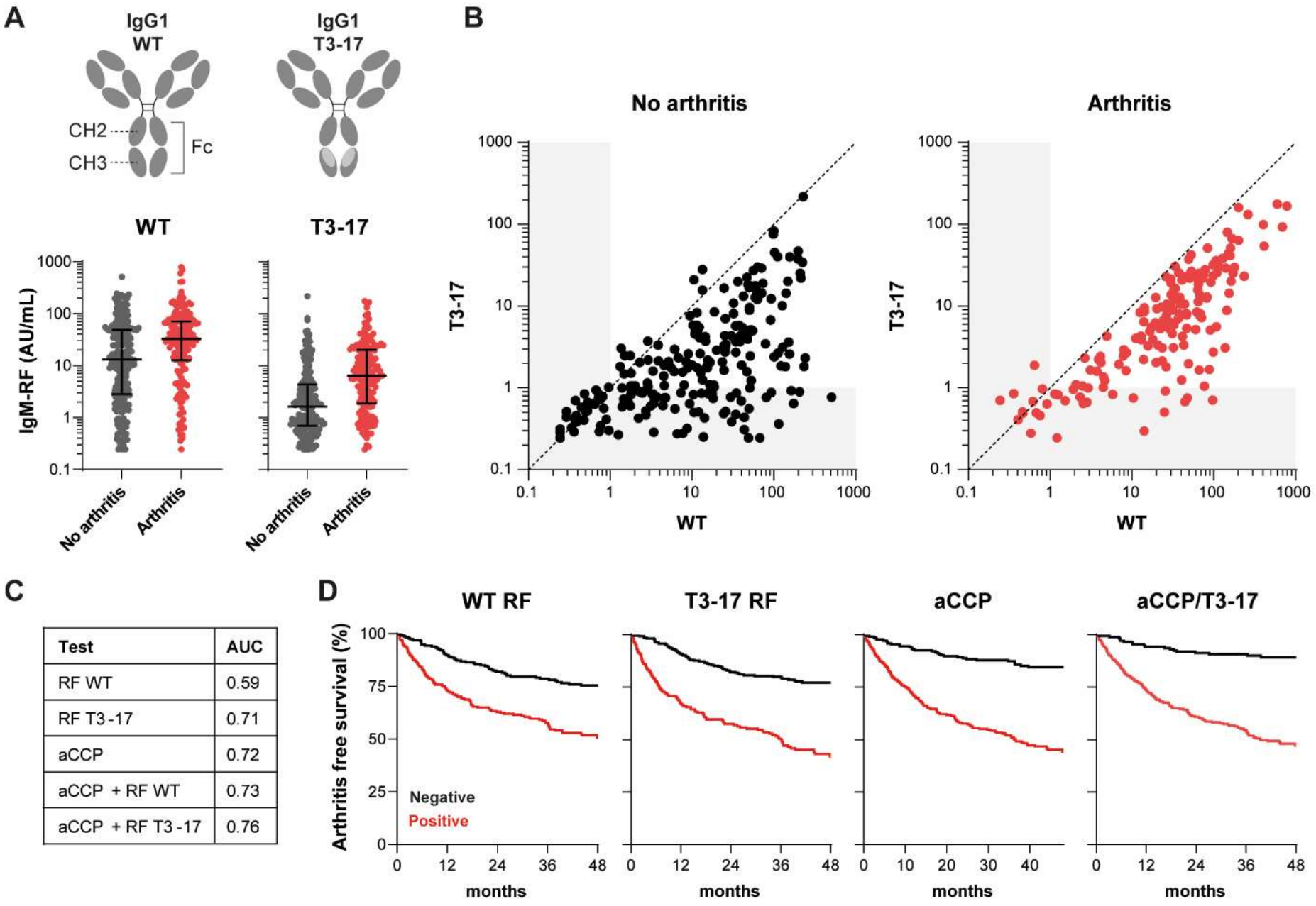
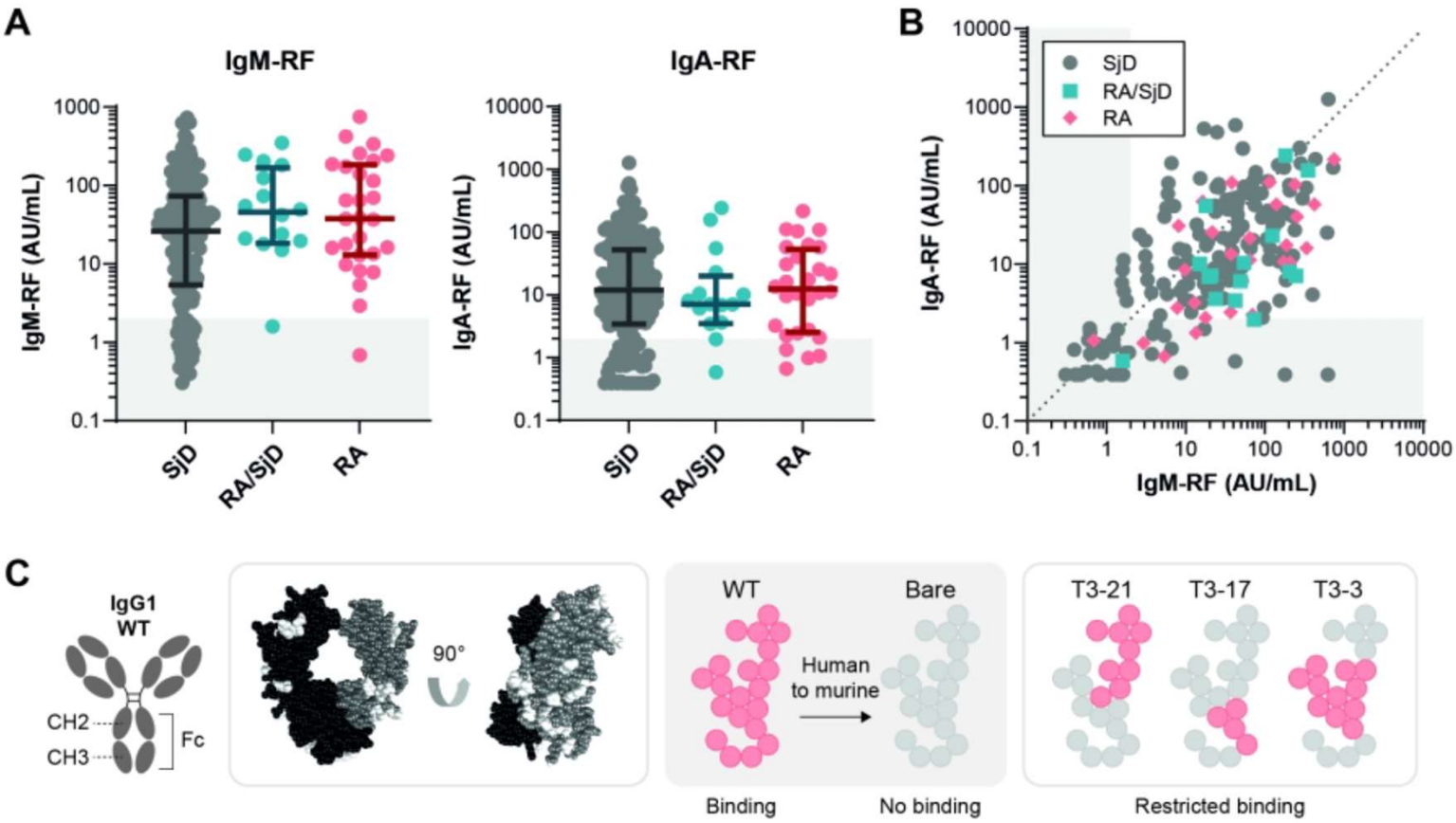
Improved predictive power in at-risk population for development of RA

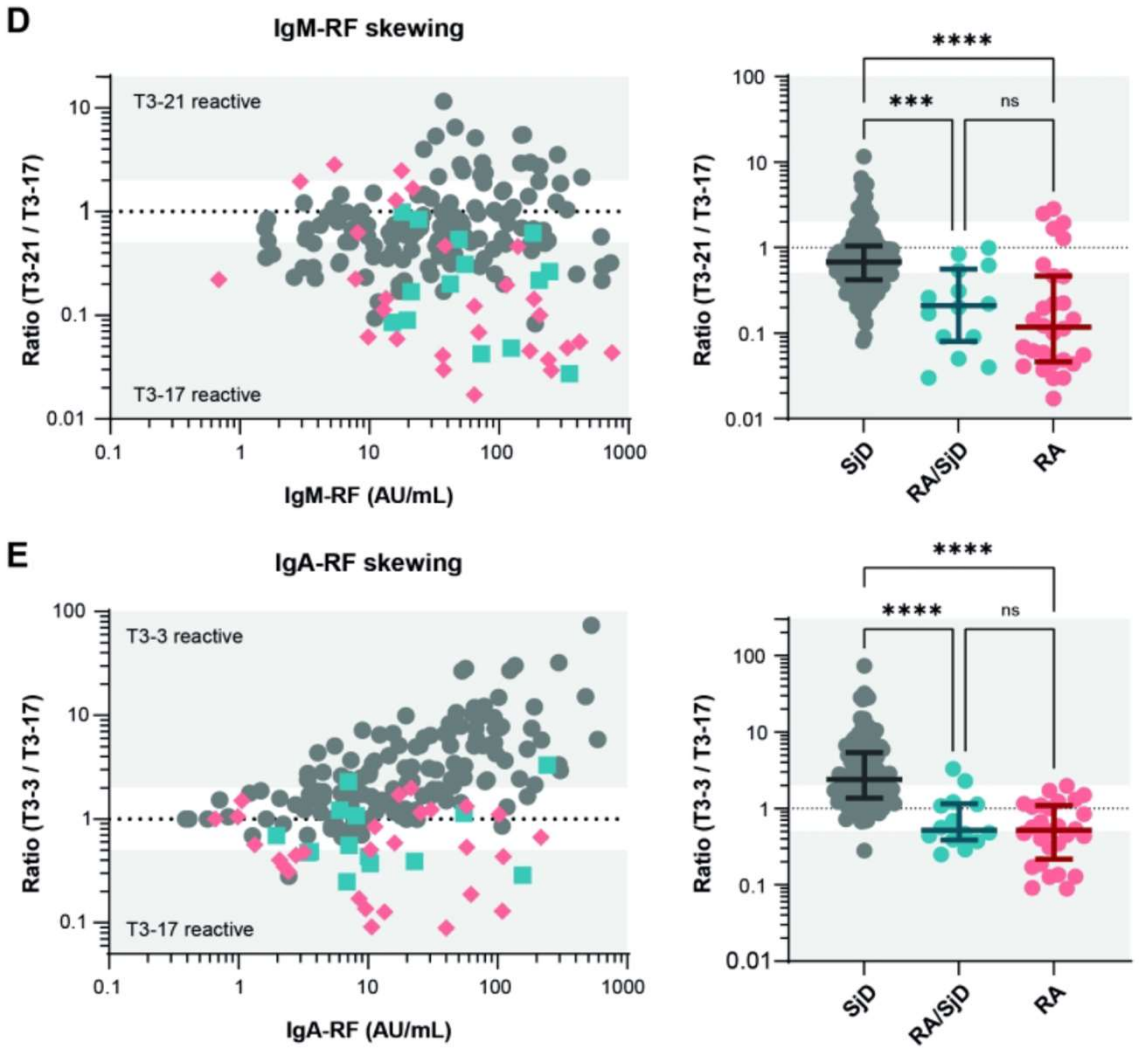


Test	AUC
RF WT	0.59
RF T3-17	0.71
aCCP	0.72
aCCP + RF WT	0.73
aCCP + RF T3-17	0.76

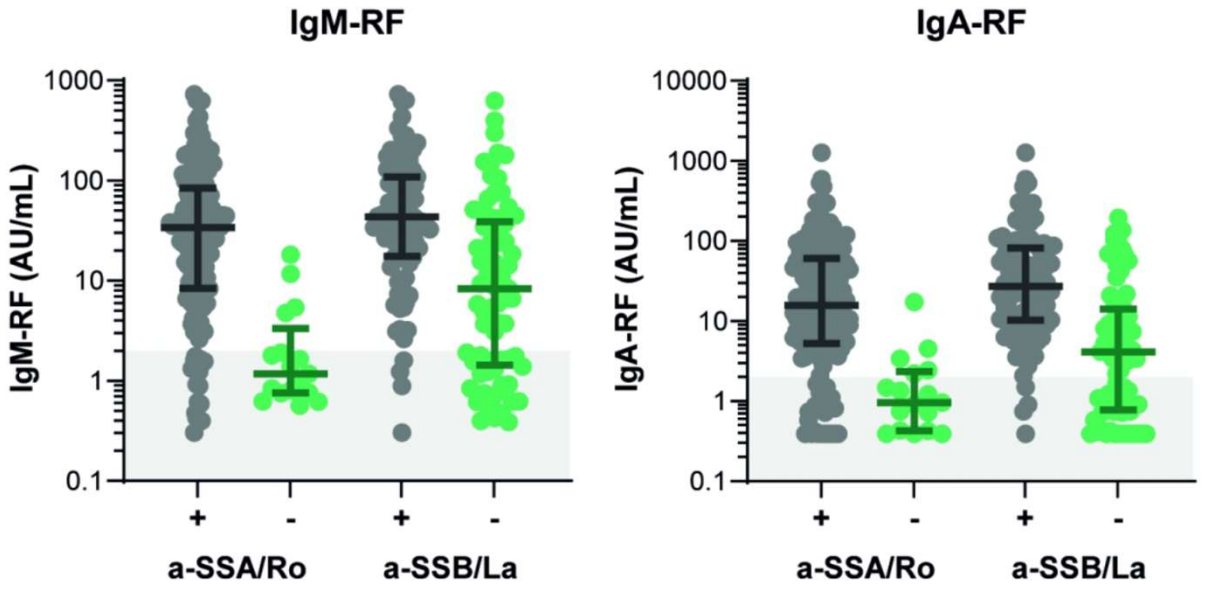
Different rheumatoid factor binding patterns distinguish between primary and RA-associated Sjögren's Disease



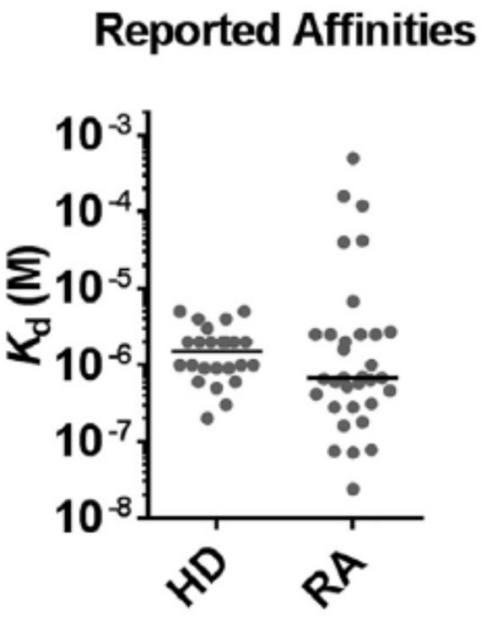
Different rheumatoid factor binding patterns distinguish between primary and RA-associated Sjögren's Disease

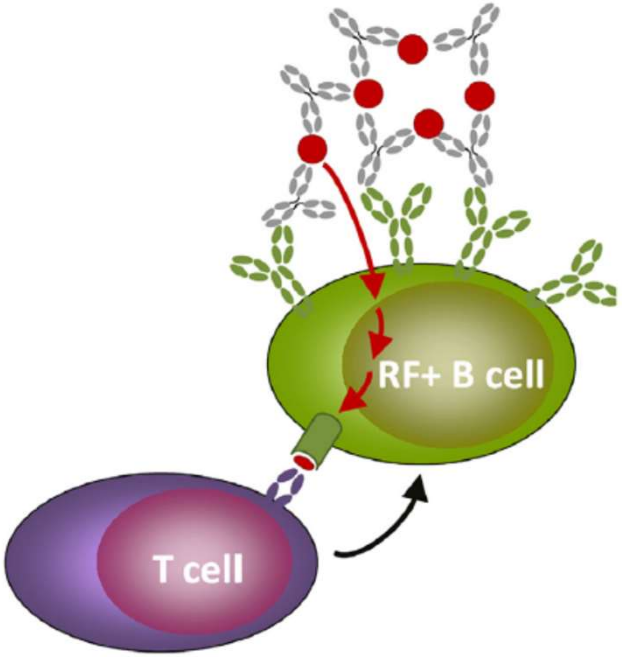


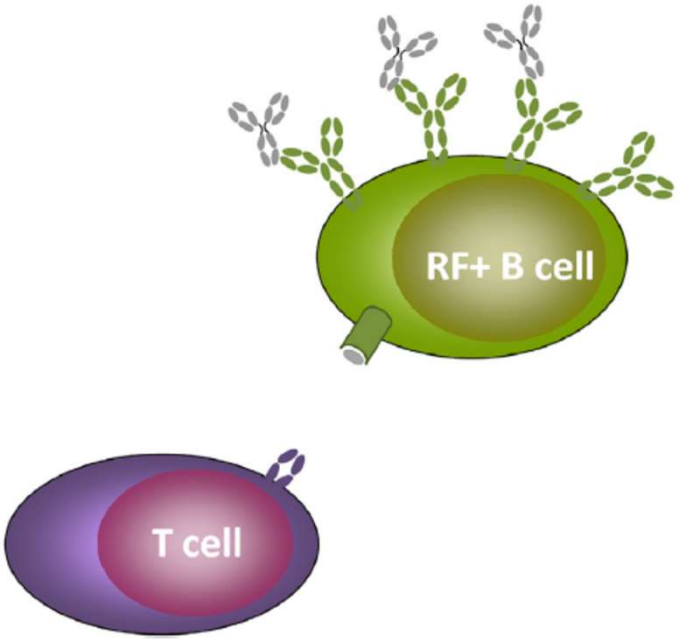
Different rheumatoid factor binding patterns distinguish between primary and RA-associated Sjögren's Disease



Rheumatoid factors: anti-IgG or anti-immune complex?

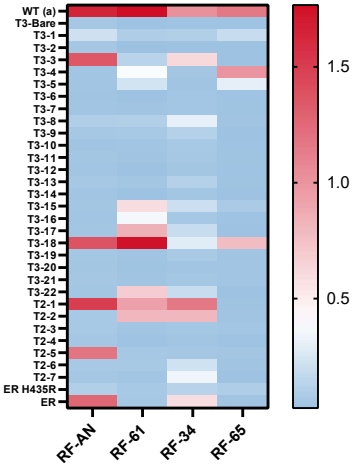


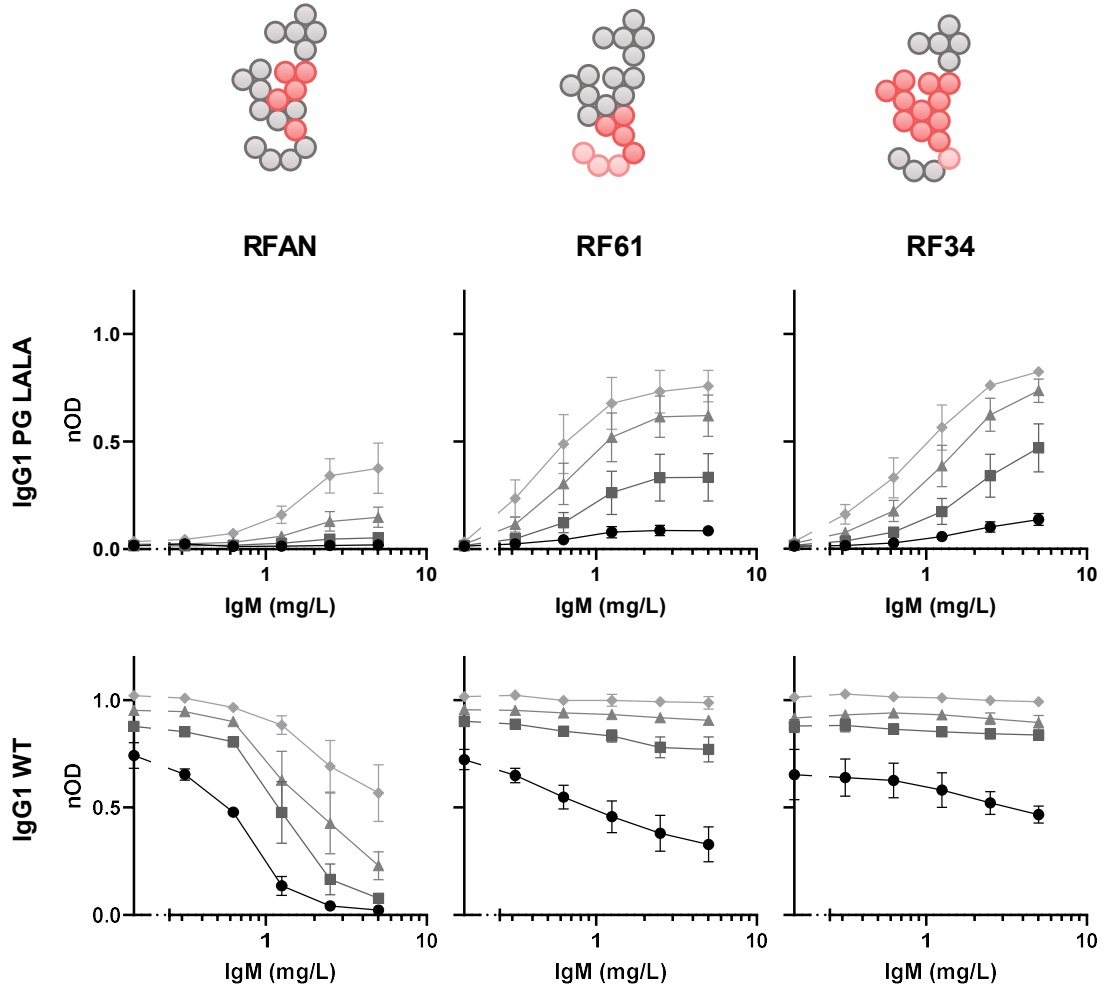




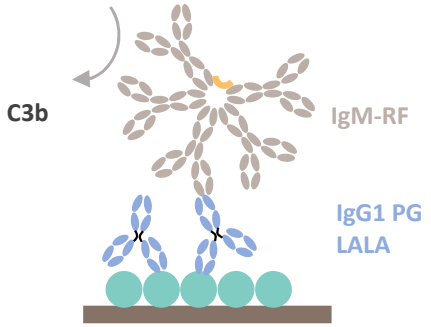
Falkenburg et al. Semin Arthritis Rheum 2018

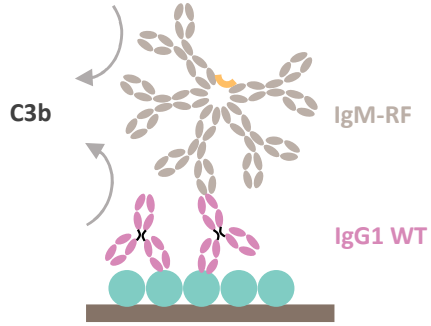
Complement activation by recombinant patient-derived IgM rheumatoid factors





Complement deposition time
- 7.5 min
- 15 min
- 30 min
- 60 min





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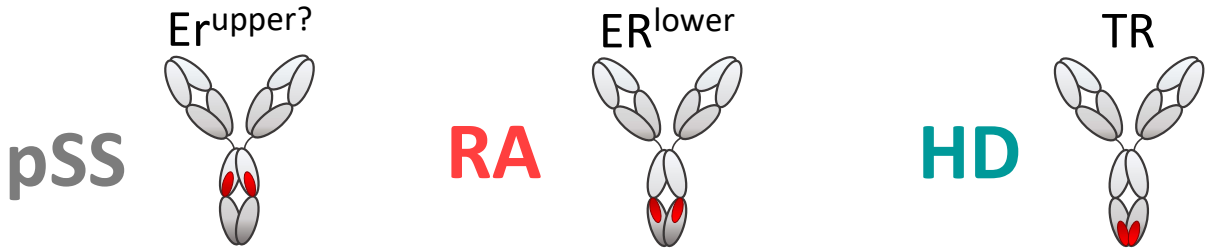
Different RFs target different epitopes on the IgG-Fc

Certain reactivities more strongly associated with pathology

Distinct patterns in RA vs pSS



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UMCG

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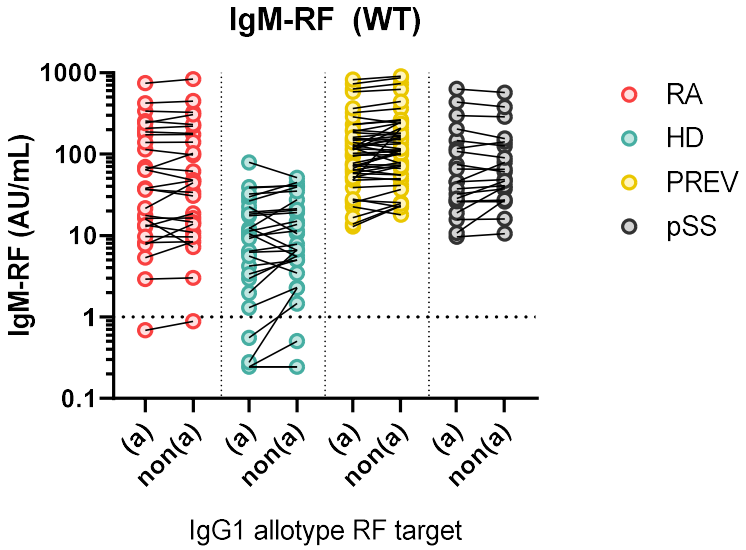
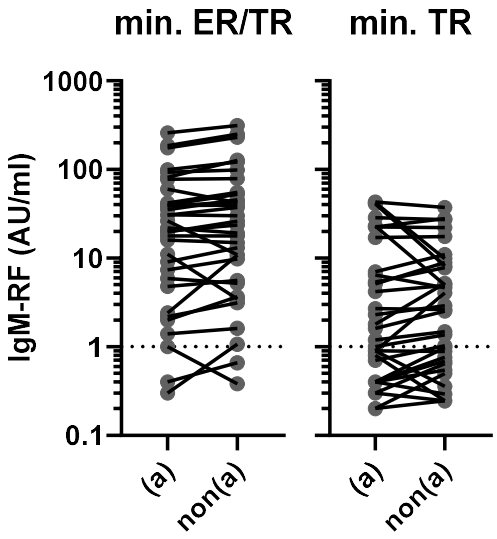
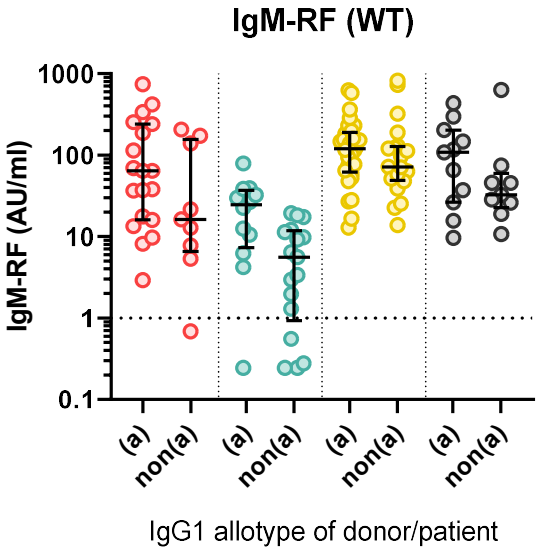
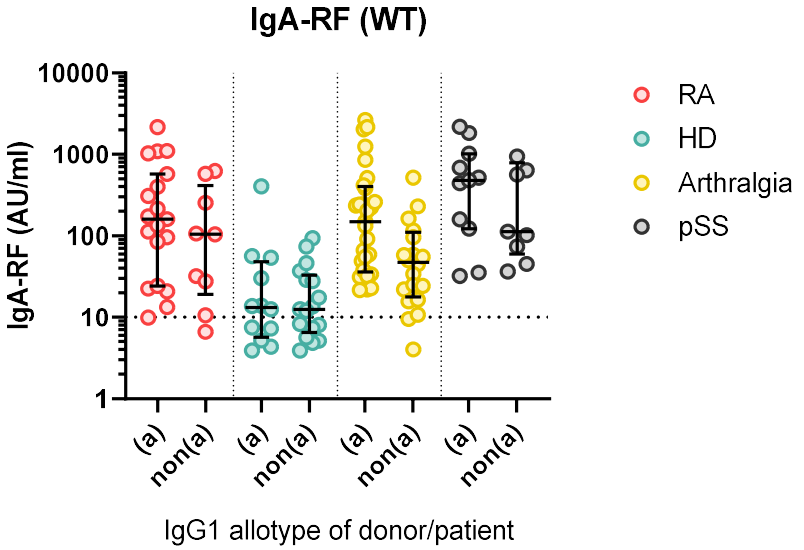
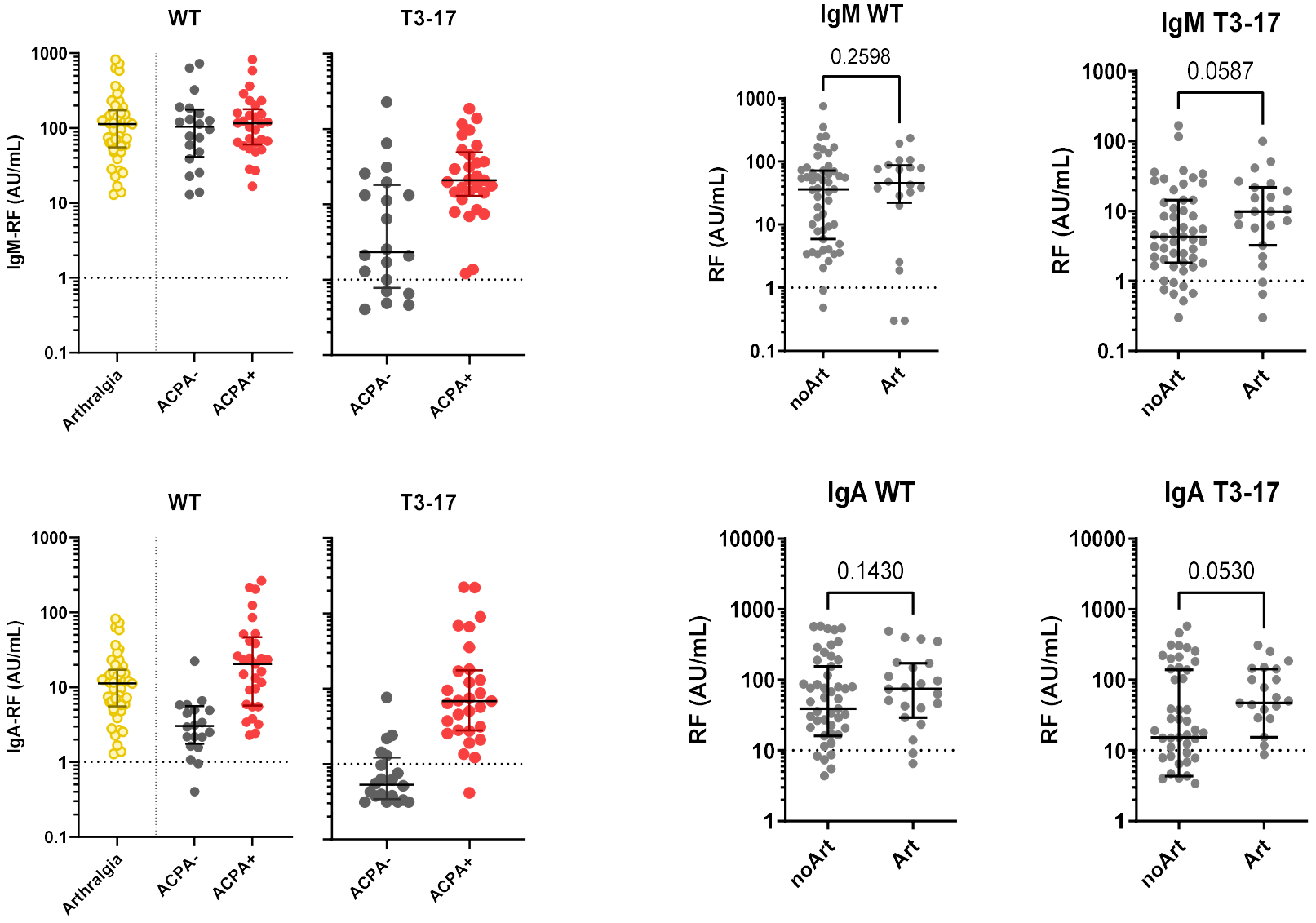
Allotypes: G1m(a) vs non(a)











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