Autoantibodies from single circulating plasmablasts react with citrullinated antigens and *Porphyromonas gingivalis* in rheumatoid arthritis

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Rheumatoid Arthritis (RA)

- A chronic, systemic, autoimmune inflammatory disorder that principally attacks synovial joints.

- Affects about 1% of the general population worldwide, women three times more often than men.

- Reduces the lifespan of patients by a range of 3 to 12 years.

Etiology of RA

- Genetic factors (50-60%):
    - HLA-DR4
    - PTPN22
    - PAD4
- Environmental factors (40-50%):
    - Smoking
    - Microbial infection (*eg. Porphyromonas gingivalis*)
   - Aberrant physiological process (apoptosis, NETosis)

Autoantibodies in RA

- *Rheumatoid factor (RF, anti-immunoglobulin Fc, 1940)

- Anti-collagen II antibody

- Anti-glucose-6 phosphate isomerase (GPI) antibody

- Antibodies to heat shock protein (HSP) antibody

- *Anti-citrullinated protein antibody (ACPA, 1999), over 90% specificity for RA

RF and ACPA are serological diagnostic criterion for RA (2010 ACR/EULAR)

Anti-citrullinated protein antibody (ACPA) in RA

• 1964 Anti-perinuclear factor antibody (APF)
• 1979 Anti-keratin antibody (AKA)
• 1995 Anti-filaggrin antibody
• 1999 Citrulline is essential for autoantibody recognizing filaggrin

• 2001 Anti-cit-fibrinogen
• 2004 Anti-cit-vimentin
• 2005 Anti-cit-collagen
• 2005 Anti-cit-α-enolase
• 2012 Anti-cit-Bip
• 2014 Anti-cit-histone
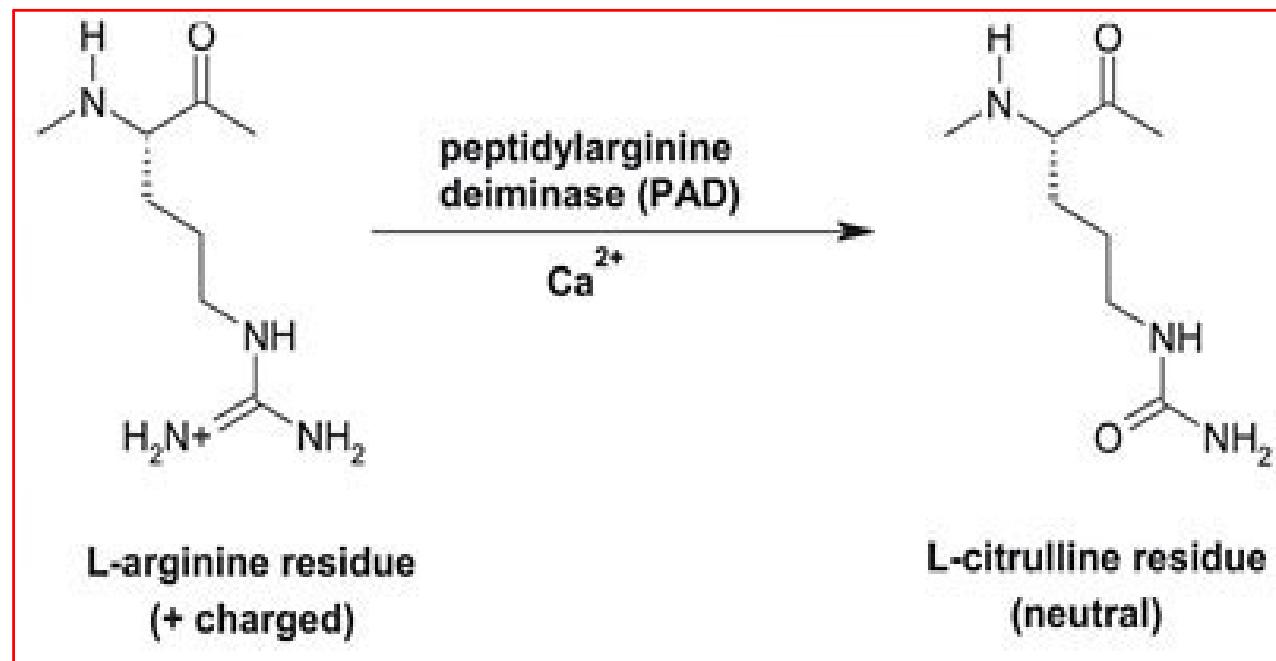
Commercial kits
1. 2001 Anti-cyclic
 citrullinated peptides(CCP) assay
2. 2004 Anti-CCP2 assay
3. 2007 Anti-mutated vimentin (AMV) assay

peptidylarginine deiminase (PAD)
Ca^{2+}

L-arginine residue (+ charged) → L-citrulline residue (neutral)

Pathogenicity of ACPAs in RA

- ACPAs precede years before the clinical diagnosis of RA and predict RA with a higher OR than RF and HLA SE.

- ACPAs identify subgroups of early RA patients with a more severe disease course.

- Passive transfer of ACPAs enhanced tissue injury in collagen-induced arthritis (CIA) mice.

- Citrullinated antigens have increased arthritogenicity in animal models of arthritis.

- ACPAs induce macrophages to secret tumor necrosis factor alpha (TNFα), a dominant inflammatory cytokine in RA.

Central questions

- **What are the molecular features of ACPAs?**
- **Where are the cells that produce ACPAs?**
- **What triggers the generation of ACPAs?**

VDJ recombination and somatic mutation contribute to antibody repertoire

Heavy chain genome sequence (V, D, J)   Light chain genome sequence (V, J)

Rearranged DNA

Germline gene

Somatic mutation

Combined binding site

An antibody is made up
of pairs of heavy and light chains

Light

Heavy

"constant region"

Heavy

Light
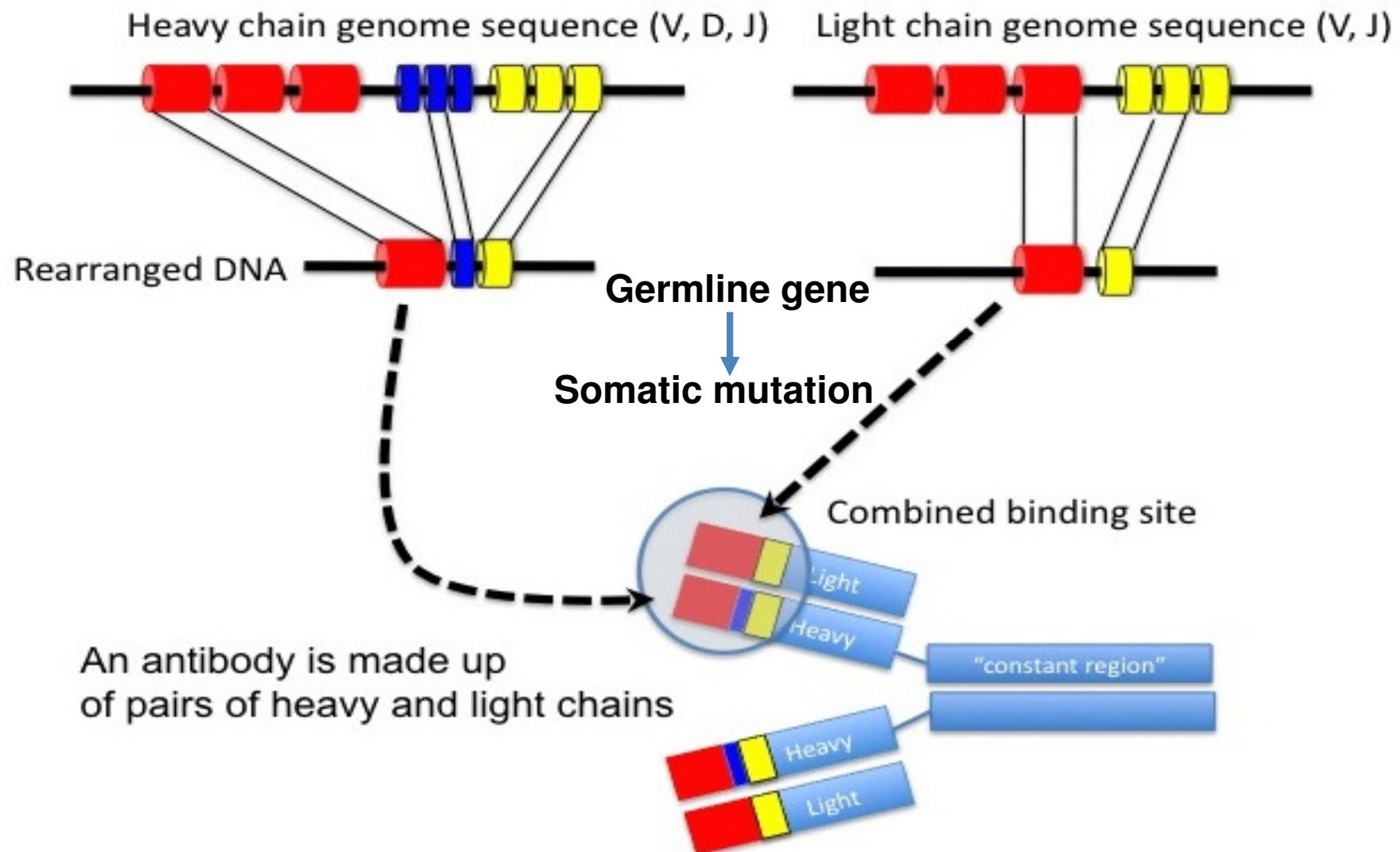
# Development of B Lymphocytes
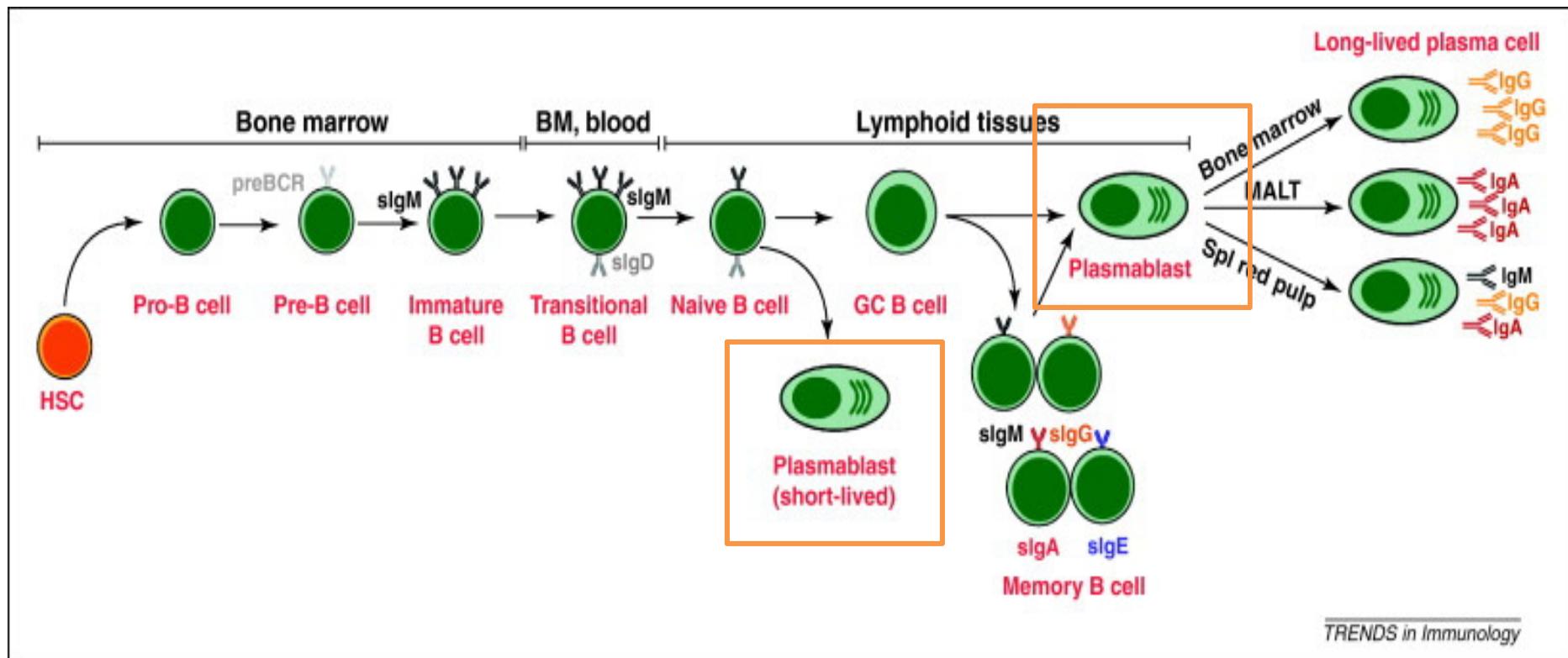
Tangve SG, *Trends in Immunol*, 2011

Frequency of circulating plasmablasts is increased in RA patients

A

Healthy control · RA patient

CD27 · CD19

B

Frequency of plasmablasts in CD19$^+$ B cells (%)

***$p < 0.001$

Control · RA
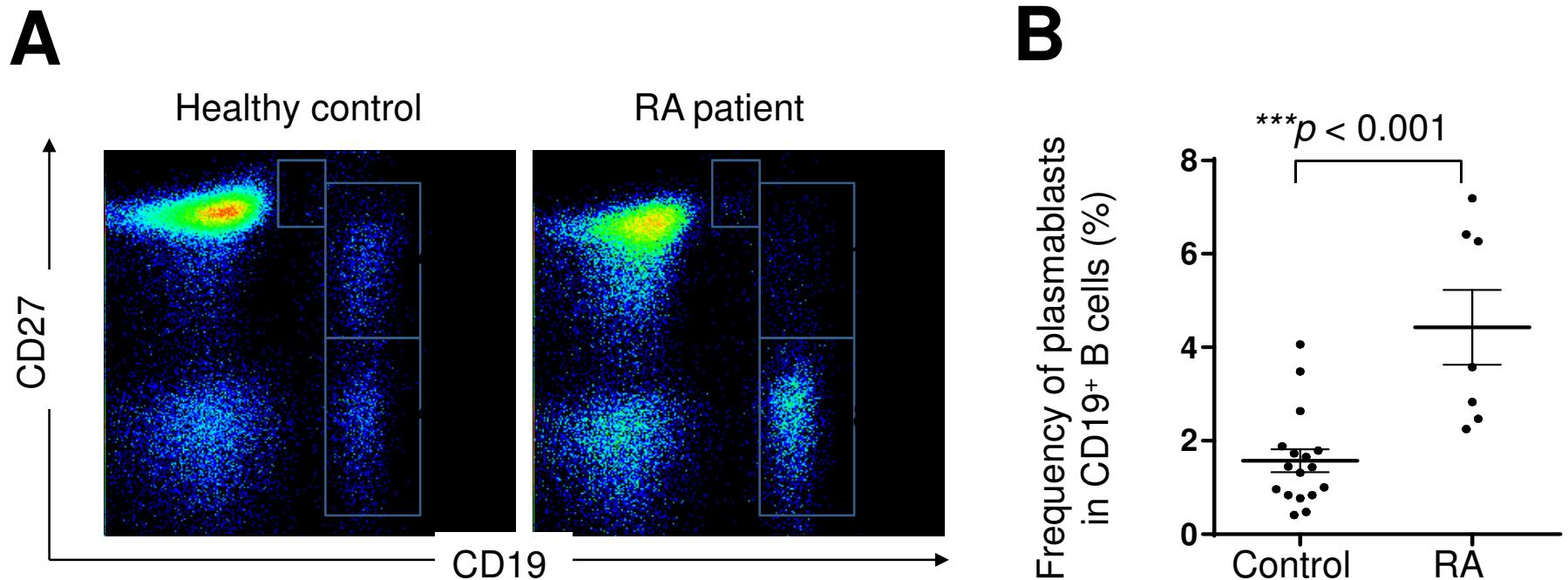
Single Cell RT-PCR

A

CD27 / CD19
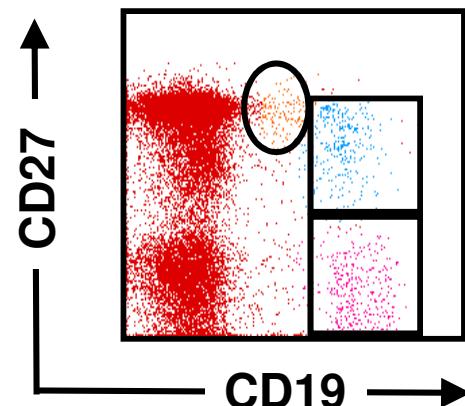
FACS
sort

Single-Cell RT PCR
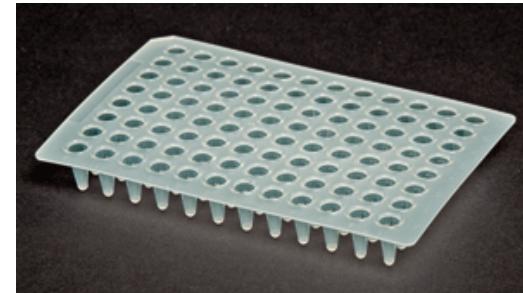
Two more rounds of PCR to
amplify IgH and IgL genes

B

CMV V$_H$DJ Cγ — γ

CMV VkJk Cκ — κ

CMV VlJl Cλ — λ
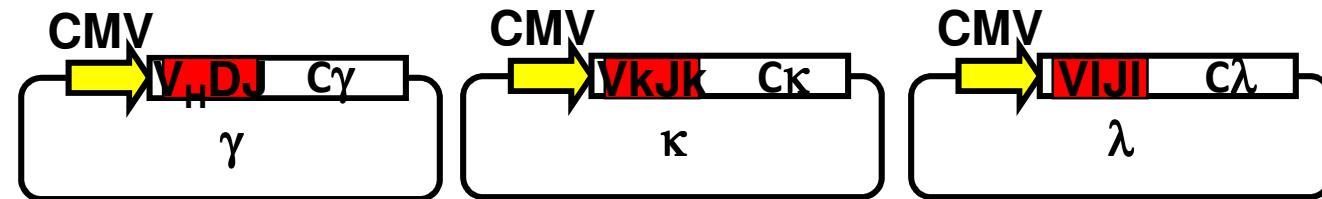
→ DNA sequencing
and analysis

293T cells
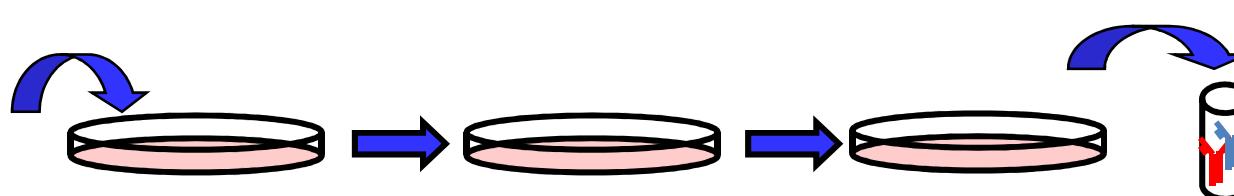
Recombinant Ab

→ ELISA to test
the reactivity
of Ab

Circulating plasmablasts in RA patients produce anti-CCP Abs


A

Healthy control (n = 4)   CCP+ RA (n = 6)   CCP- RA (n = 1)

CCP2 OD$_{450}$

	U95	U110	U111	U113	RA78	RA70	RA79	RA40	RA97	RA89	RA88
					20.7%	4.8%	15.2%	18.2%	8.3%	3.2%	0%
					(6/29)	(1/21)	(5/33)	(6/33)	(4/48)	(1/31)	(0/22)

Cut-off for positivity

CCP2+ / total **0.0% (0/107)**

11.8% (23/195)

***$P < 0.001$

B

Frequency of CCP2+ antibodies (%)

*$p < 0.05$, $R^2 = 0.548$

Serum anti-CCP2 (RU/mL)
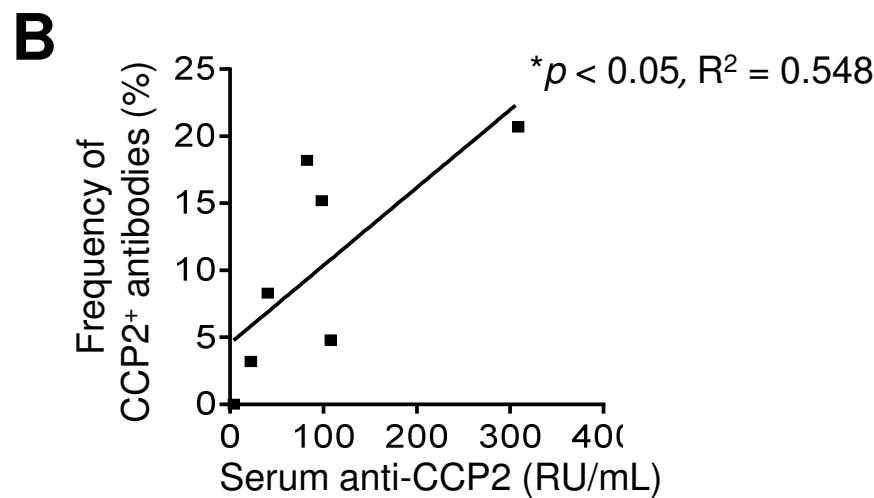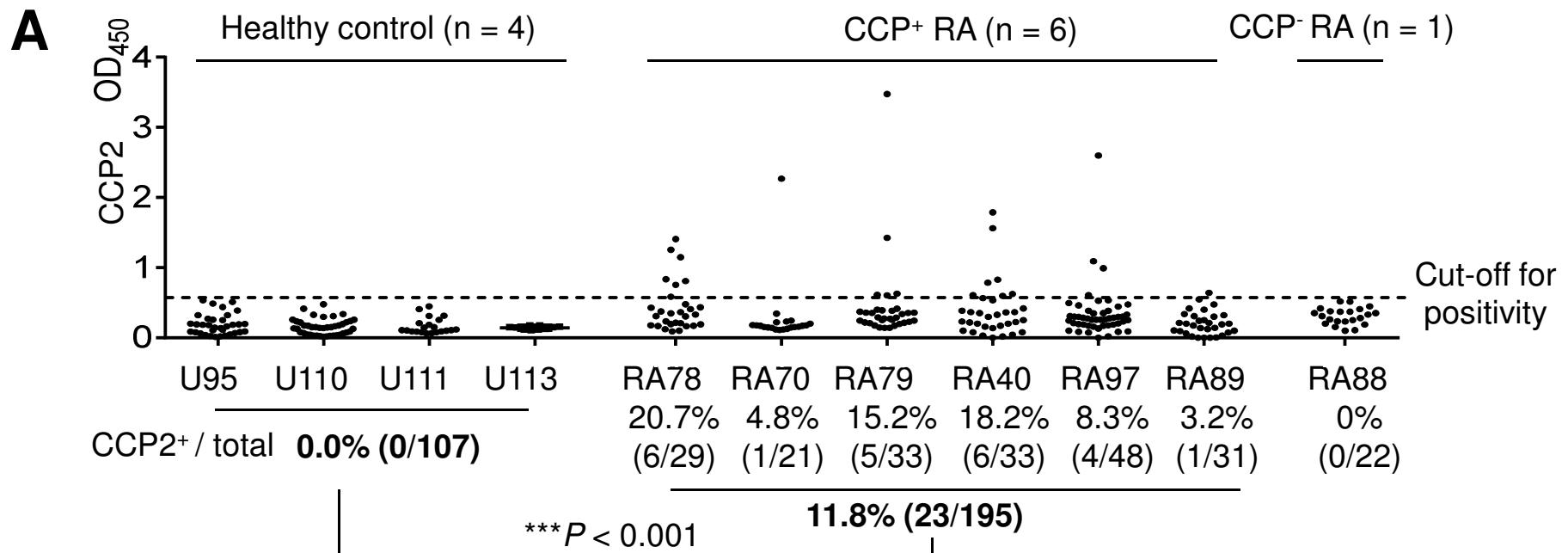
Fine specificity of ACPAs

A

Healthy Control | RA78 | RA70 | RA79 | RA40 | RA97 | RA89 | RA88

cFib, cEno, cVim (OD$_{450}$ vs µg/mL: 0.05, 0.33, 1.25, 5.00)

0%
(0/107)
cFib/Eno/Vim+

| 31.0% | 4.8% | 27.3% | 21.2% | 18.8% | 3.2% | 0% |
| (9/29) | (1/21) | (9/33) | (7/33) | (9/48) | (1/31) | (0/22) |

18.5% (36/195) cFib/Eno/Vim+

B

Control | RA78 | RA70 | RA79 | RA40 | RA97 | RA89 | RA88

Legend: CCP2$^+$ only | cFib/Eno/Vim$^+$ only | CCP2$^+$ and cFib/Eno/Vim$^+$

Control: 107 / 107

RA78: 2, 7, 29 / 20
RA70: 1, 21 / 20
RA79: 5, 4, 33 / 24
RA40: 1, 2, 5, 33 / 25
RA97: 5, 4, 48 / 29
RA89: 1, 1, 31 / 29
RA88: 22 / 22

0%
(0/107)
ACPA$^+$

31.0%	4.8%	27.3%	24.2%	18.8%	6.5%	0%
(9/29)	(1/21)	(9/33)	(8/33)	(9/48)	(2/31)	(0/22)
$p < 0.0001$	$p = 0.1641$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.05$	$p = 1.0000$

19.5% (38/195) ACPA$^+$

****$p < 0.0001$

The generation of ACPA is antigen-driven
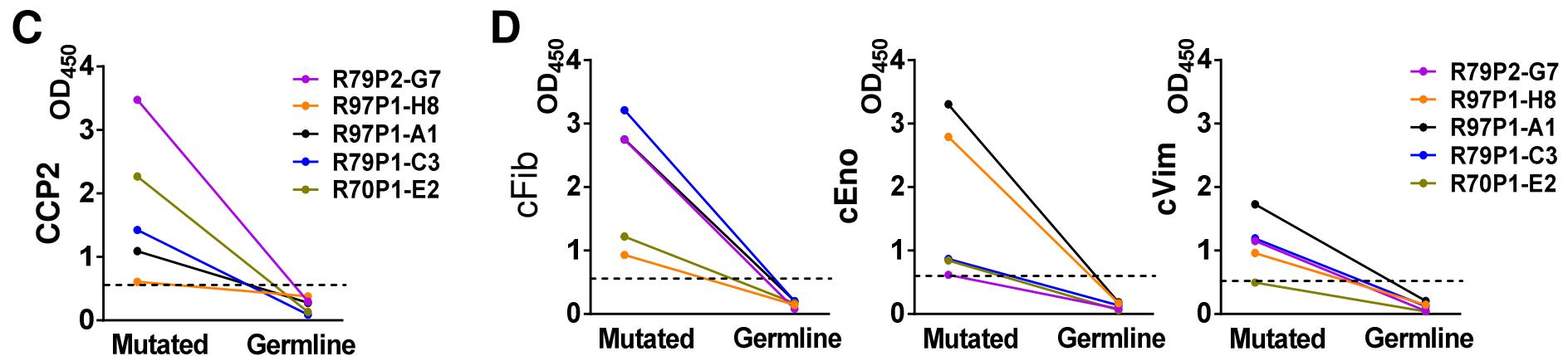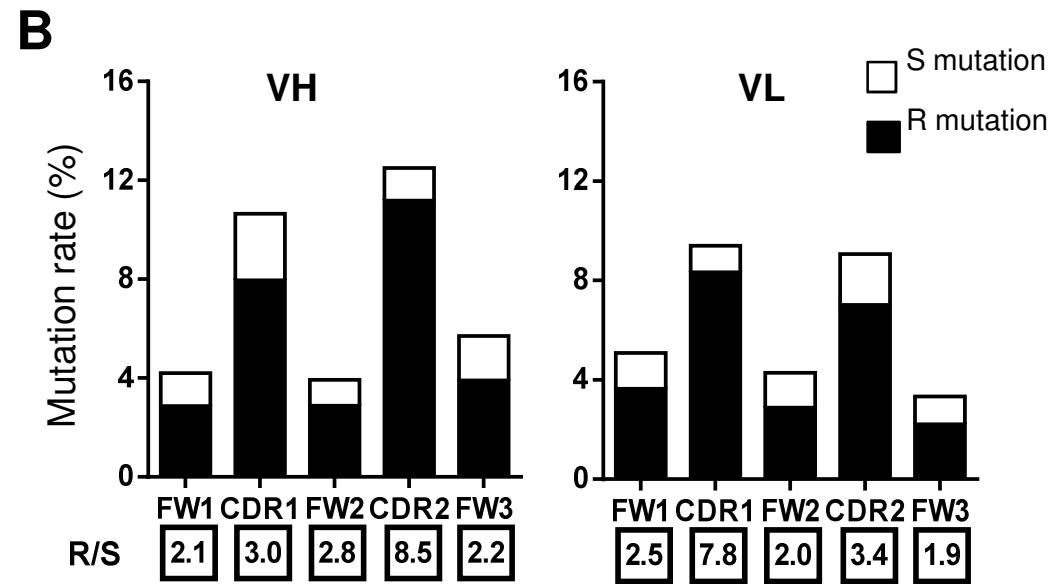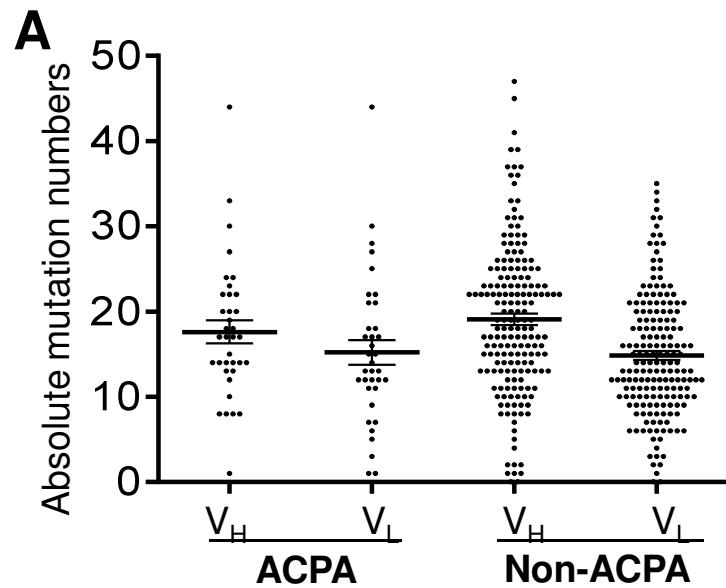
RA patient-derived ACPAs react with *P. Gingivalis* antigens

A

ACPA (n = 38) — 39.5% (15/38)
Non-ACPA (n = 43) — 2.3% (1/43)
Control Abs (n = 43) — 0% (0/43)

B

ACPA (1/38)   Non-ACPA (1/43)   Control (0/43)

C

ACPA (0/38)   Non-ACPA (1/43)   Control (0/43)
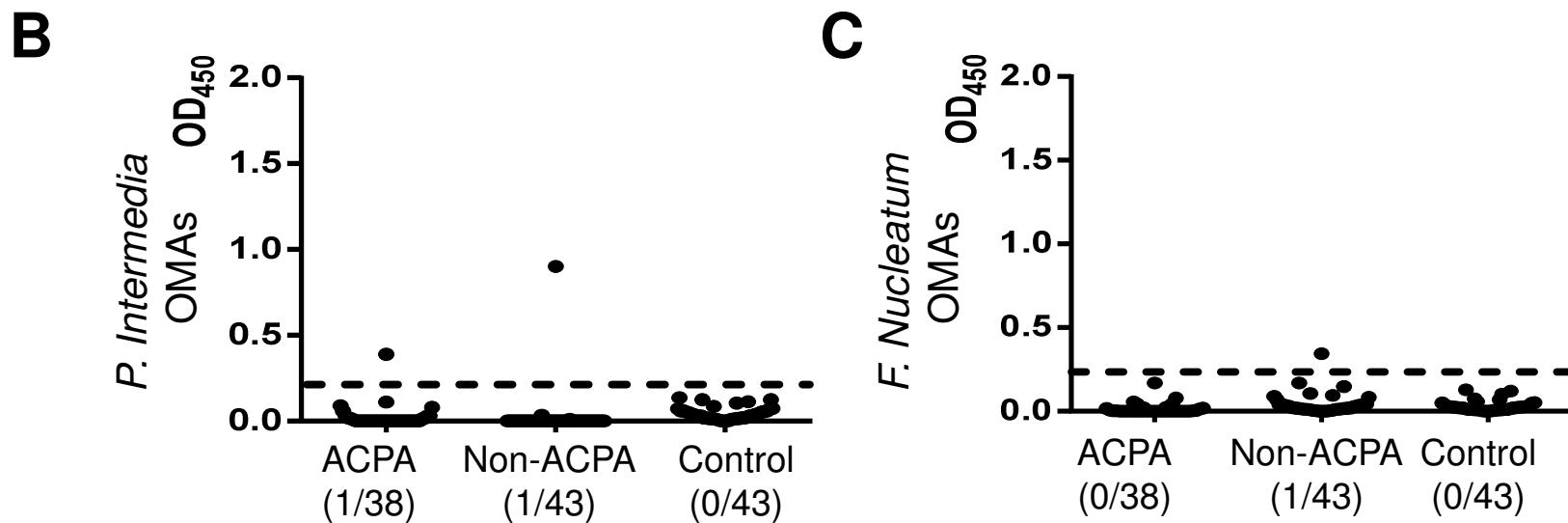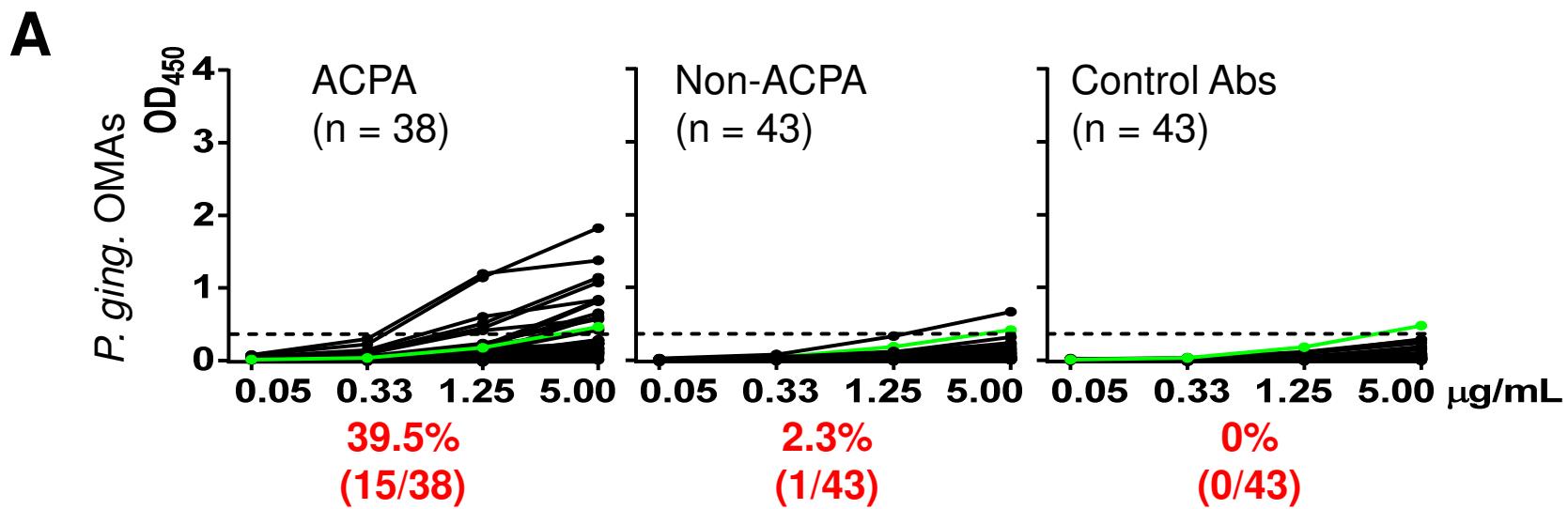
RA patient-derived ACPAs react with citrullinated *P. Gingivalis* enolase
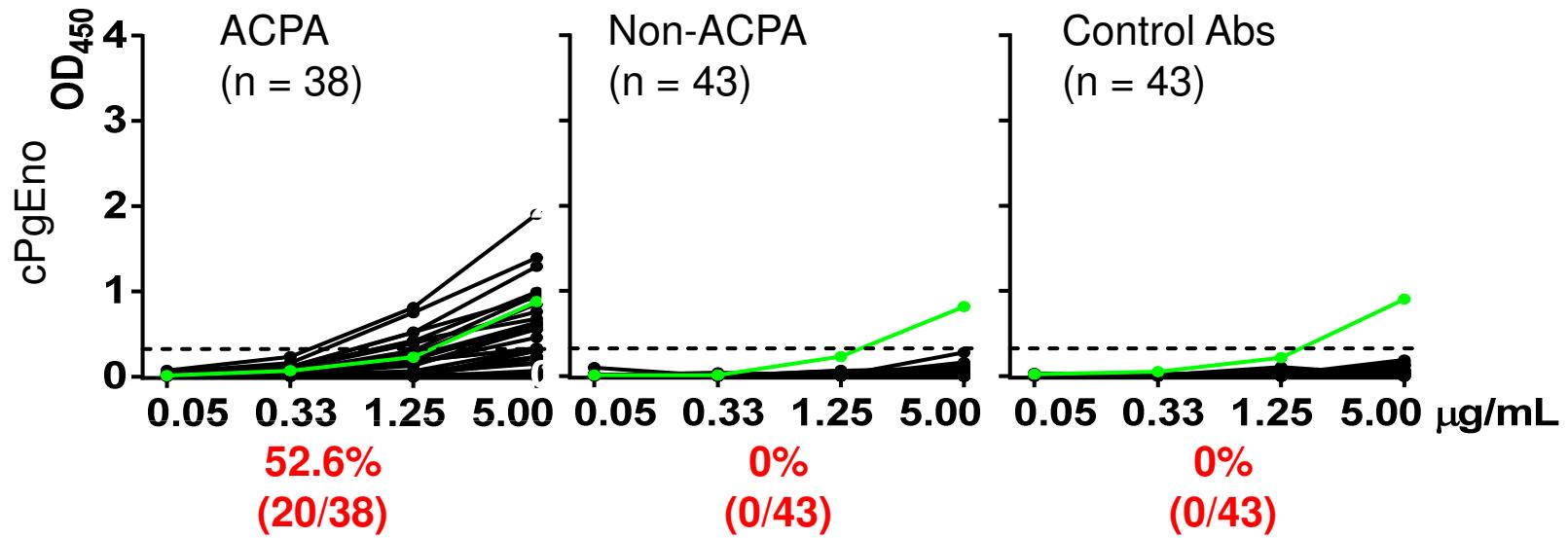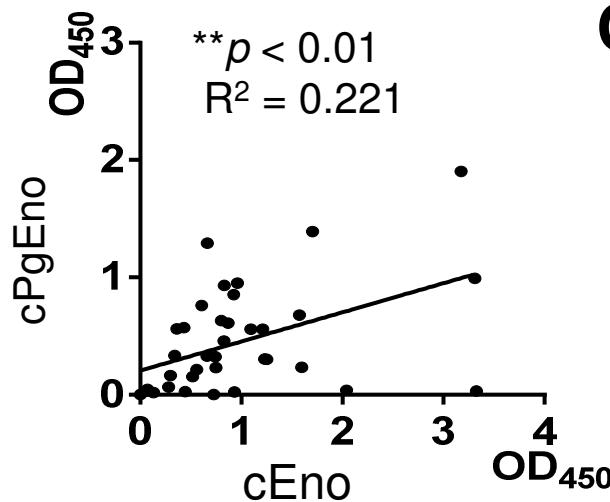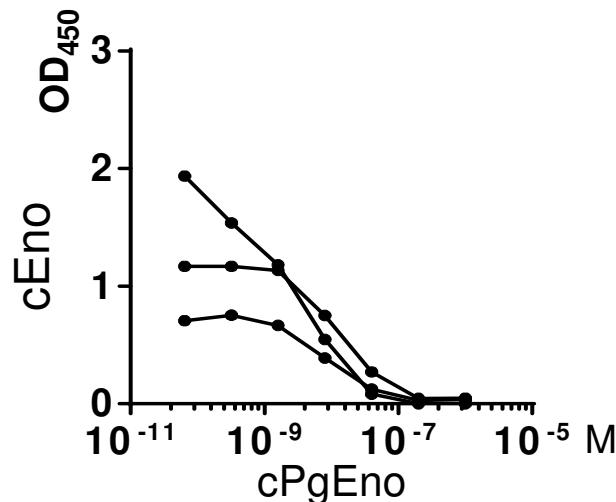
The generation of ACPAs may be initiated by anti-*P. Gingivalis* responses
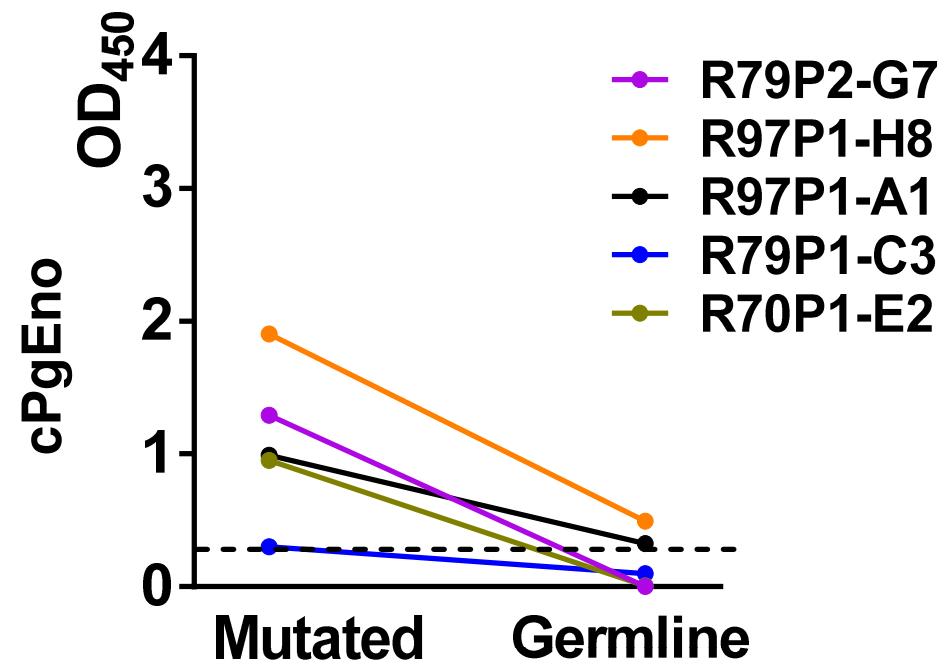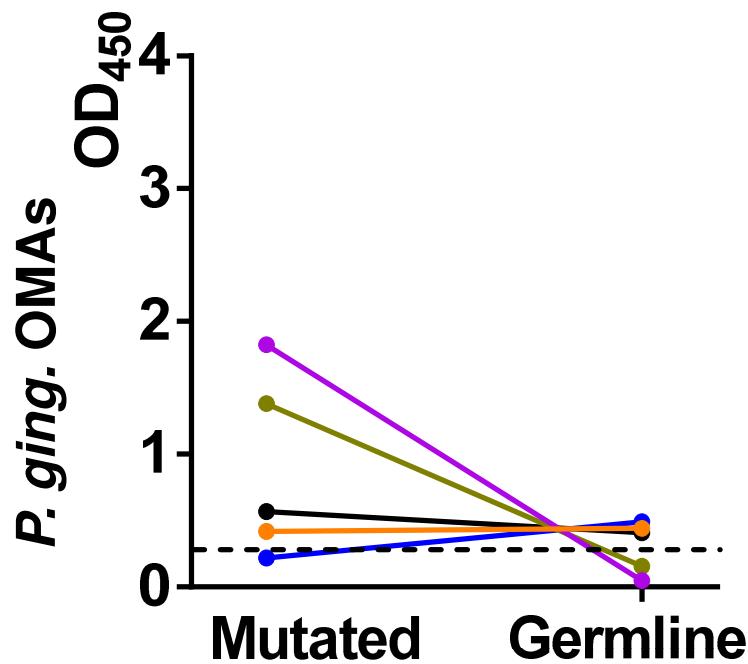
Summary

1. Circulating plasmablasts from serological CCP$^+$ RA patients preferentially express ACPAs ($\sim$20% ranging from 5-31% in CCP$^+$ RA vs 0% in CCP$^-$ RA and healthy controls).

2. The reactivities of RA patient-derived ACPAs are generated by somatic hypermutation.

3. The evolvement of ACPA-encoding B cells in RA patients is an antigen-driven process.

4. RA patient-derived ACPAs, but not non-ACPAs or control antibodies, react with *P. Gingivalis* antigens.

5. Anti-*P. Gingivalis* immune responses in RA patients may initiate the generation of ACPAs.

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